Transmittal Letter to the United States Designated/Elected Office (DO/EO/US)

Prepared from FORM PTO-1390

JC10 Rec'd PCT/PTO 16 JAN 2002

Attorney's Docket No.	: M38-025
U.S. Application No.	: Not Yet Assigned
U.S. Application Filed	: Herewith
International Application No.	: PCT/IB00/00966
International Filing Date	: 14 July 2000 (14.07.00)
Priority Date Claimed	: 20 July 1999 (20.07.99)
Title of Invention	: PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS
Applicants for (DO/EO/US)	: VITOLONE, Vincenzo; LOTTI, Andrea; and GOTTARDI, Massimo

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. _X_ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ___ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ___ This express request to begin national examination procedures [35 U.S.C. 371 (f)] at any time rather than delay examination until the expiration of the applicable time limit set forth in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. _X_ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. _X_ A copy of the International Application as filed [35 U.S.C. 371(c)(2)]
   a) ___ is transmitted herewith (required only if not transmitted by the International Bureau);
   b) _X_ has been transmitted by the International Bureau;
   c) ___ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ___ A translation of the International Application into English [35 U.S.C.371(c)(2)].
7. _X_ Amendments to the claims of the International Application under PCT Article 19/34 [35 U.S.C.371(c)(3)]
   a) ___ are transmitted herewith (required only if not transmitted by the International Bureau);
   b) _X_ have been transmitted by the International Bureau, and have been made a part of the specification as filed;
   c) ___ have not been made; however, the time limit for making such amendments has **NOT** expired;
   d) ___ have not been made and will not be made.
8. ___ A translation of the amendments to the claims under PCT Article 19 [35 U.S.C.371(c)(3)].
9. _X_ An oath or declaration of the inventor(s) [35 U.S.C.371(c)(4)].
10. ___ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 [35 U.S.C.371(c)(5)].

EXPRESS MAIL No.: **EL 890535414 US** Deposited: **January 16, 2002**

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: Commissioner of Patents and Trademarks, BOX PCT, Washington, D. C. 20231.

Curtis L. Schrandt — January 16, 2002

Curtis L. Schrandt — Date

Items 11. to 16. below concern other document(s) or information included:

11. ___ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. _X_ An Assignment document for recording. (A separate Cover Sheet in compliance with 37 CFR 3.28 and 3.31 is included.)
13. _X_ A **FIRST** preliminary amendment.
    ___ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. _X_ A substitute specification.
15. ___ A change of power of attorney and/or address letter.
16. _X_ *(other items or information)* Published International Application Publication No. WO 01/06251 A3 with International Search Report; Abstract (attached to Substitute Specification and Claims); International Preliminary Examination Report; and Verified Statement Claiming Small Entity Status.

17. _X_ The following fees are submitted:

		CALCULATIONS	PTO USE ONLY
	BASIC NATIONAL FEE [37 CFR 1.492(a)(1)-(5)]:		
_X_	Search Report has been prepared by the EPO or JPO $ 890.00	$890.00	
___	International preliminary examination fee paid to USPTO [37 CFR 1.482] $ 710.00		
___	No International preliminary examination fee paid to USPTO [37 CFR 1.482] but International search fee paid to USPTO [37 CFR 1.445(a)(2)] $ 740.00		
___	Neither International preliminary examination fee [37 CFR 1.482] nor International search fee [37 CFR 1.445(a)(2)] paid to USPTO $ 1040.00		
___	International preliminary examination fee paid to USPTO [37 CFR 1.482] and all claims satisfied provisions of PCT Article 33(1)-(4) $ 100.00		
	ENTER APPROPRIATE BASIC FEE AMOUNT	$890.00	
	Surcharge of $130.00 for furnishing the oath or declaration later than ___20 / _X_30 months from the earliest claimed date [37 CFR 1.492(e)]		
	Claims: Number filed / Number extra / Rate		
	Total Claims **14** -20 = 0 x $ 18. =	$	
	Indep. Claims **1** - 3 = 0 x $ 84 =	$	
	Multiple Dependent Claim(s) (if applicable) + $ 280. =	$	
	TOTAL OF ABOVE CALCULATIONS =	$ 890.00	
	Reduction by ½ for filing by **small entity**, if applicable. Verified Small Entity Statement must be filed. [Note 37 CFR 1.9, 1.27, 1.28] -	$ 445.00	
	SUBTOTAL =	$445.00	
	Processing fee of $130.00 for furnishing the English Translation later than ___20 / ___30 months from the earliest claimed priority date [37 CFR 1.492(f)] +	$	
	TOTAL NATIONAL FEE =	$ 445.00	
	Fee for recording the enclosed assignment [37 CFR 1.21(h)]. The assignment must be accompanied by an appropriate cover sheet [37 CFR 3.28, 3.31]. $40.00 per property +	$ 40.00	
	TOTAL FEE (ENCLOSED) =	**$485.00**	
	(AMOUNT TO BE REFUNDED OR CHARGED) REFUNDED	$	
	CHARGED	$	

a) √ A check in the amount of **$485.00** to cover the above fees is enclosed.
b) __ Please charge my Deposit Account No. **04-0838** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.
c) √ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **04-0838**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 36 CFR 1.494 or 1.495 has not been met, a petition to revive [37 CFR 1.137(a) or (b)] must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

R. Neil Sudol
Coleman Sudol Sapone, P.C.
714 Colorado Avenue
Bridgeport, CT 06605-1601
Tel. (203) 366-3560

R. Neil Sudol	R Neil Sudol	31,669	January 16, 2002
Name	Signature	Reg. No.	Date

Applicant/Patentee: AZIENDA PROVINCIALE PER I SERVIZI SANITARI
Serial/PatentNo.: _____
Filed/Issued: _____
For: PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS
Attorney's Docket No. : _____

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

( ) the owner of the small business concern identified below:

(X) an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN AZIENDA PROVINCIALE PER I SERVIZI SOCIOSANITARI
ADDRESS OF CONCERN Via De Gasperi, 79
38100 TRENTO - ITALY

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled:
PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS
by inventor(s) VITALONE, Vincenzo; LOTTI, Andrea; GOTTARDI, Massimo
described in

(X) the specification filed herewith.
( ) application serial no. _____, filed _____.
( ) patent no. _____, issued _____.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).
*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).

COLEMAN SUDOL SAPONE, P.C.
714 Colorado Avenue
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(203) 366-3560

Cont'd.

Verified Statement (Declaration) Claiming Small Entity Status
(37 CFR 1.9(f) and 1.27(c)) - Small Business Concern

Applicant/Patentee : AZIENDA PROVINCIALE PER I SERVIZI SANITARI
Serial/Patent No. : _____
Filed/Issued : _____
For : PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS
Attorney's Docket No. : _____

NAME OF CONCERN _____

ADDRESS OF CONCERN _____

( )INDIVIDUAL ( )SMALL BUSINESS CONCERN ( )NONPROFIT ORGANIZATION

I acknowledge the duty to file in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patents issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING FAVARETTI, Carlo
TITLE OF PERSON OTHER THAN OWNER General Director
ADDRESS OF PERSON SIGNING via delle Orne n.9 - 38100 TRENTO

SIGNATURE _____
DATE 15 GEN. 2002

COLEMAN SUDOL SAPONE, P.C.
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IN THE UNITED STATES RECEIVING OFFICE (DO/EO/US)

Applicants	: VITOLONE, Vincenzo; LOTTI, Andrea; and GOTTARDI, Massimo
U.S. Application No.	: Not Yet Assigned
U.S. Application Filed	: Herewith
Int'l Application No.	: PCT/IB00/00966
Int'l Filing Date.	: 14 July 2000 (14.07.00)
Priority Date Claimed	: 20 July 1999 (20.07.99)
Title of Invention	: PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS

Commissioner for Patents
Box PCT
Washington, D.C. 20231

PRELIMINARY AMENDMENT

S I R:

Prior to calculation of the filing fee in the above-identified application, please amend the application as follows:

IN THE CLAIMS:

Amend claim 2 as follows:

2. (Once Amended) Process according to claim 1, wherein said solid sample is a sample of hair.

Amend claim 3 as follows:

3. (Once Amended) Process according to claim 1, wherein said temperature is ranging from 100°C to 150°C.

Amend claim 4 as follows:

4. (Once Amended) Process according to claim 1, wherein said period of time is ranging from 15 minutes to 24 hours.

Amend claim 5 as follows:

5. (Once Amended) Process according to claim 1, wherein said temperature is maintained at 100°C for 1 hour.

Amend claim 10 as follows:

10. (Once Amended) Process according to claim 1, wherein the analyzed samples are arranged in increasing order of concentration of cocaine or other alkaloids.

Amend claim 11 as follows:

11. (Once Amended) Process according to claim 1, wherein the samples are subjected to confirmation analyses with standard techniques such as GC or GC/MS.

Amend claim 12 as follows:

12. (Once Amended) Process according to claim 2, wherein each hair sample is made of about 50mg to 300mg of finely divided and/or powdered hair.

Amend claim 14 as follows:

14. (Once Amended) Diagnostic kit for carrying out the process according to claim 1, comprising a liquid reagent with constant concentration of hydroxyl groups suitable for extracting cocaine and other alkaloids and transforming cocaine into benzoylecgonine, and a conventional screening kit for the determination of said metabolite in urine samples.

REMARKS

Claims 1 through 14 are pending in the application. The present amendment is submitted to eliminate multiple dependent claims, particularly improper multiple dependent claims, and to reduce the filing fee.

Respectfully submitted,

COLEMAN SUDOL SAPONE, P.C.

By: _____

R. Neil Sudol
Reg. No. 31,669

Dated: 16 Jan. 2002

714 Colorado Ave.
Bridgeport, Connecticut 06605-1601
203-366-3560

APPENDIX TO PRELIMINARY AMENDMENT
AMENDED CLAIMS IN U.S. NATIONAL PHASE OF PCT/IB00/00778

2. (Once Amended) Process according to claim[s] 1, wherein said solid sample is a sample of hair.

3. (Once Amended) Process according to claim[s] 1 [and 2], wherein said temperature is ranging from 100°C to 150°C.

4. (Once Amended) Process according to claim[s] 1 [and 2], wherein said period of time is ranging from 15 minutes to 24 hours.

5. (Once Amended) Process according to [any preceding] claim[s] 1, wherein said temperature is maintained at 100°C for 1 hour.

10. (Once Amended) Process according to [any preceding] claim[s] 1, wherein the analyzed samples are arranged in increasing order of concentration of cocaine or other alkaloids.

11. (Once Amended) Process according to [claim any preceding] claim[s] 1, wherein the samples are subjected to confirmation analyses with standard techniques such as GC or GC/MS.

12. (Once Amended) Process according to [any preceding] claim[s] 2, wherein each hair sample is made of about 50mg to 300mg of finely divided and/or powdered hair.

14. (Once Amended) Diagnostic kit for [the] carrying out [of] the process according to [any] claim[s] 1 [to 13], comprising a liquid reagent with constant concentration of hydroxyl groups suitable for extracting cocaine and other alkaloids and transforming cocaine into benzoylecgonine, and a conventional screening kit for the determination of said metabolite in urine samples.

Substitute Specification, Including Amendment Made

Under PCT Article 34 to Specification and Claims

Title of Invention:

PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS

International Application Number: PCT/IB00/00966
International Filing Date: 14 July 2000 (14.07.2000)
International Publication Number: WO 01/06251 A3
International Publication Date: 25 January 2001 (25.01.2001)

10/031423
JC13 Rec'd PCT/PTO 16 JAN 2002
WO 01/06251 PCT/IB00/00966

16/prts

PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS.

This invention relates to a process for the quantitative determination of cocaine and other alkaloids, such as morphine and methadone, in a solid sample, e.g., a sample of hair, by using a screening type approach.

The invention also relates to a reagent for use in such process and new diagnostic kits including such reagent among their components.

By "screening type approach" is meant a kind of analysis permitting to analyze in a relatively short time span a relatively large number of samples in a cheap, efficacious and standardized manner. This kind of analysis permits to exclude the negative samples by immediately identifying the samples that do not contain the substance or the entire substance class or those in which said substances are present at a level lower than the threshold or cut-off value.

The threshold or cut-off is a practical limit selected to establish if the sample analyzed is positive or negative. The threshold value differs from the limit of detection of the method, that is, the lowest concentration of an analyte that can be determined. In fact, the cut-off value is normally set at a concentration higher than the limit of detection in order to obviate the imprecision of the analysis at values close to the limit of detection.

Cut-off values are conventionally established and take into account a multiplicity of factors, such as the capability of using commercially available reagents, the pharmacokinetic properties of the substances and the need to avoid false negatives (see: P.Zuccaro, S.Pichini, I.Altieri and R.Pacifici: Proposta di linee guida per l'analisi delle sostanze d'abuso nei liquidi biologici

in RAPPORTI ISTISAN 96/29 edited by ISTITUTO SUPERIORE DI SANITA').

The substances that the metabolism of the human body accumulates in the hair are numerous. Usually, it is required to detect the presence of alkaloids and other substances of abuse, such as, morphine, methadone and/or cocaine. By the method of this invention further substances can also be detected, such as, those of the following non-limitative list: 6-O- mono-acetyl-morphine, bi-acetyl-morphine, codeine, papaverine, nalorphine, nicotine, cotinine, caffeine, noscarpine, mepivacaine, trimetropin, buprenorphine, pentazocyne, methadone metabolyte, benzoylecgonine, amphetamine, metamphetamine, methylenedioxyamphetamine, methylenedioxymetamphetamine, methylenedioxyethylamphetamine, benzodiazepines, barbiturates.

Description of the state of the art

Several techniques are known for the determination of the analytes of interest, such as those mentioned above. Specifically, for the analysis of cocaine that is present in hair, gas chromatography (GC) combined with Mass Spectrometry (MS) hereinafter referred to as GC/MS and Radio Immune Assay technique (R.I.A.) are known.

GC or liquid chromatography (LC) combined with MS are methods of resolution, purification or separation and identification of components of complex mixtures of organic or inorganic substances having even strictly similar chemical properties. The separation of substances dissolved in a liquid or fixed on a finely divided or porous solid substance is based on percolation, respectively elution trough them of an eluent gas, respectively liquid.

When the substances to separate/detect can be made gaseous and as eluent a

gas is used, GC applies. The latter is a separation method based on the distribution between a solid or liquid stationary phase and a mobile phase made of the gases or vapors to separate, which are carried by a stream of an inert gas.

The analytical results are reported in a graph named chromatogram, in which the quantities of the single components present in the mixture and transferred to the eluent gas/liquid are reported versus time. The graph has peaks whose highness is a direct function of the quantity of the specific substance.

Chromatographic methods that can be used for confirmatory analyses are GC and LC, the latter being often referred to as High Performance Liquid Chromatography (HPLC). The most commonly employed detectors for GC are electron scatting detectors and phosphor nitrogen detectors.

As said above, GC/MS employs a gas chromatograph coupled to a mass spectrograph. By so doing, the separation capability of GC combines with the specificity proper of MS. Therefore, GC/MS represents the method of choice for confirmatory analyses of the above named substances as well as their metabolites (see again "Proposta di linee guida per l'analisi delle sostanze d'abuso nei liquidi biologici" by ISTITUTO SUPERIORE DI SANITA')

In the conventional acid hydrolysis, however, cocaine is extracted by hydrochloric acid as it is, i.e. without undergoing transformation into its metabolite benzoylecgonine. Since the amounts of cocaine extracted in this way are very little, it is not possible to determine cocaine by the usual screening methods.

Radio Immune Assay (RIA) is based on radio-immunological tests using known amounts of antibodies and of analyte labeled with a radioisotope (generally

I^{125}). During incubation the labeled analyte and that possibly present in the sample compete for the antibody sites. After precipitation of antigen-antibody complexes and centrifugation the supernatant or the precipitate are transferred to a gamma Geiger counter that measures the radioactivity level. RIA kits are extremely sensitive and allow identification of 1-5 ng of substance per ml. Adoption of automatic instruments for pipetting and counting allows the contemporaneous analysis of numerous samples with response times of 1 to 5 hours. On the other hand, the use of radioactive isotopes requires adequate safety measures. Furthermore, the relatively short half-life of the radioactive isotopes imposes a careful handling of reagents.

RIA does not lend itself to a widespread use for the quick determination of the substances in question in view, first of all, of the high cost of reagents, further increased by liability of the antisera. Secondly, in view of the criticality of the analysis due to the need of operating with radioactive materials that are dangerous for the analysts and also in view of the required times which are relatively long. RIA also requires the availability of rooms properly equipped and shielded, as well as particular care in handling waste materials and in their disposal.

Therefore, RIA is scarcely employed in this sort of analyses. The prior art shows therefore the impracticality of determining cocaine present in the hair by the so-called screening type approach, as described above, i.e., by very quick and cheap techniques as are used for the determination of the same substances in urine. This is mainly due to the fact that in the conventional screening-type approach what is analyzed is not cocaine, but its metabolite benzoylecgonine. The fact that this substance does not exist in nature by itself, but only as the metabolyte of cocaine and the fact that the reaction of transformation of cocaine into benzoylecgonine, i.e. the transformation of the ester group into an hydroxyl group, is irreversible in an alkaline environment

render the determination of benzoylecgonine a necessary and sufficient condition for the detection of cocaine.

Benzoylecgonine is found in the hair in a ratio of 1 to 4 or even 1 to 10 and more with respect to cocaine. Therefore, when the amount of cocaine is very low or close to the cut-off values (0.2-0.1 ng cocaine per mg hair) screening cannot be performed because of the low amount of this metabolyte (0.05-0.025 ng benzoylecgonine per mg hair), such amount being undetectable by the screening apparatuses available in the laboratories.

US-A-5 910 419 which is the nearest prior art to the invention relates to a method for screening hair samples using the well known ELISA technique for the presence of cannabinoids and further RIA for the presence of cocaine. As already outlined, the radio immunoassay does not lend itself to quick and cheap determination of drug of abuse and further involves safety and handling difficulties.

Summary of the invention

The main object of this invention is to overcome the above mentioned drawbacks, i.e. the impossibility to dose the cocaine present in a solid sample by the conventional screening-type approach.

In accordance with the invention, this object is achieved by a screening-type procedure for the quantitative determination of cocaine and other alkaloids which are present in a solid sample which, in accordance with claim 1, includes the following steps:

a) preparing a solid sample in a finely divided or powdered form;

b) selecting a liquid reagent providing constant concentration of

hydroxyl groups suitable for extracting and transforming cocaine into benzoylecgonine and for extracting other similar substances;

c) extracting cocaine and other similar substances contained in the sample and transforming the extracted cocaine into benzoylecgonine by maintaining the sample completely immersed in said liquid reagent at a temperature ranging from 10°C to 250°C for a period of time ranging from few seconds to 48 hours; and

d) analysing the liquid separated from the solid sample to determine the concentration of benzoylecgonine contained in said liquid with respect to the cut-off limit using a conventional screening kit for the determination of the said substance in urine.

Preferred aspects of the invention include using hair as the solid material forming the solid sample; using a buffer as the reagent which provides hydroxyl groups, more preferably an ammonia buffer, most preferably a buffer that is hereinafter referred to as VMA; and heating the sample immersed in the liquid at a temperature of about 100 to 150°C for about one hour.

In accordance with another aspect of the invention a process is provided which includes the additional steps of:

- arranging samples by increasing concentrations of the substances of interest; and
- performing confirmation analyses with known techniques, such as, GC or GC/MS.

In one embodiment of the invention, the process may provide the following steps:

- providing a sample made of about 50 to 300 mg of finely divided and/or powdered material;

- adding in the test tube containing the said sample a suitable liquid reagent until the sample is completely immersed, said reagent being capable of performing extraction and transformation of cocaine into benzoylecgonine and at the same time of extracting other similar substances which are present in the sample
- if necessary, agitating the test tube to facilitate immersion of the sample;
- heating the contents of the test tube to a temperature T1 for a time interval t1 by keeping the test tube immersed in a thermostated bath or by placing it in an oven;
- cooling the test tube to room temperature;
- taking the liquid and transferring it into a test tube suitable for a screening type instrument;
- performing the screening by using a kit of reagents for the determination of the said substances in urine;
- reading the data resulting from the first level instrumentation to verify the concentration values with respect to the cut-off limit; and

- contemporaneously determining the amount(s) of substance(s) present.

In accordance with another aspect of the invention a reagent is provided for use in the above mentioned process which in the most preferred embodiment has the following formula:

$$VMA = 0.2\ M\ (NH_4)_2HPO_4 + 5ml/L\ 25\%\ NH_4OH$$

In the above formula $(NH_4)_2HPO_4$ is dibasic ammonium phosphate and NH_4OH is ammonium hydrate. In fact, 5ml/L NH_4OH give a 0.07 M concentration of hydroxyl groups, which is comprised in the range giving 100% conversion.

In addition to the main advantage of the invention as outlined above, another advantage is that the confirmation analyses (e.g., GC/MS) need to be performed only on those samples that have been determined to be positive at the initial screening whereas, when conventional techniques are used, all samples must be analyzed since there is no kit available for determination of cocaine, but only for determination of benzoylecgonine.

In average, the analysis of 25 samples requires two hours for the preparation of the samples plus one week for the confirmation analyses of all (both positive and negative) samples.

On the other hand, by applying the present invention, an analyst will require for the same 25 samples two hours for samples preparation plus 30 minutes for the screening-type analysis plus the time necessary for performing the confirmation analyses. However, the latter need to be performed only on those samples which have been determined to be positive.

Since, in the average, positive samples are between 0 and 20% and since

most of the time required is spent in the confirmation analyses, the procedure according to the present invention allows saving of substantial time which is estimated at about 70 to 90% of the time required for the analysis of the 25 samples taken into consideration.

Another advantage according to this invention is that the possible dragging of the active substances from one sample to another in the confirmation analyses is eliminated or minimized because it is possible to arrange samples in order of increasing concentration of the substances of interest, therefore proceeding to confirmation analyses starting from those samples having the lowest concentration and then with those having higher and higher concentrations.

In this way, it is completely eliminated the possibility that a sample with high concentration of, e.g. cocaine, leaves a trace of it in the instrument used for the analysis and has an impact on the determination of the same substance in the following sample, perhaps having a concentration just below the cut-off.

Still another advantage of this invention is that it allows to search and determine at the same time and in the same sample not only cocaine but also other substances, such as, morphine by applying the same cut-off limit or else methadone by modifying the limit of cut-off.

One further advantage is that the procedure according to this invention offers a higher guarantee to the analyst because positive outcome is confirmed by two different analytical methods.

In a further aspect of the invention a diagnostic kit is provided including the reagent described above as one of its components.

Still further additional advantages will appear from the reading of the following detailed description and the following non-limitative examples.

Brief Description of the Drawings

Figure 1 is a chromatogram obtained from an apparatus performing gas chromatography on the sample of Table 2;

Figure 2 is the graph obtained by Mass Spectrometry on the first sample after acid hydrolysis and shows presence of cocaine;

Figure 3 is the Mass Spectrum of the same first sample after treatment according to the present invention and shows presence of benzoylecgonine;

Figure 4 is the Mass Spectrum of the same first sample after treatment according to the present invention and shows presence of morphine;

Figure 5 is a chromatogram similar to that of Figure 1 and refers to the first sample treated according to this invention as shown in Table 3;

Figure 6 is similar to the MS of Figure 3 showing presence of benzoylecgonine;

Figure 7 is similar to the MS of Figure 3 showing presence of morphine;

Figure 8 is similar to Figure 1 but relates to a second sample;

Figure 9 is similar to Figure 5 but relates to a second sample;

Figures 10 and 11 are respectively similar to Figures 8 and 9 but relate to a

third sample;

Figures 12 and 13 are respectively similar to Figures 8 and 9 but relate to a fourth sample;

Figure 14 is a diagram showing transformation of cocaine into its metabolite benzoylecgonine;

Figure 15 is a graph showing concentration of cocaine versus concentration of OH^- when the reaction of transformation of cocaine into benzoylecgonine occurs at 100° C for 1 hour; and

Figure 16 is similar to Figure 15 and shows the case in which the reaction occurs at 150°C for 1 hour.

Detailed description of invention embodiments

The process of this invention substantially is a process for the quantitative extraction and transformation of cocaine into benzoylecgonine and for the extraction of similar alkaloids, in particular toxic substances of abuse and/or drugs, which are present in a sample, prepared starting from a solid material.

In the following detailed example, the sample is obtained starting from finely divided hair.

The procedure allows, in particular, the dosage of cocaine by a screening type technique, at the same time allowing the dosage of possible other toxic substances and the like present in the hair, by using kits of reagents available in commerce and intended for the analysis in urine.

The following steps are carried out:

- crush hair in fragments of two-three mm length;
- weigh the fragments to form a sample having a weight of 50 to 300 mg;
- wash the sample with methanol in a closed test tube at room temperature to eliminate possible external substances that might interfere with the results, such as, for example, traces of drugs external to the hair that have deposited on it because of its presence in the air (by so doing, it is possible to distinguish if the hair was that of a handler or that of the consumer of the drug);
-Repeat the step of washing with methanol, if necessary;
- wash the sample with ethanol in a closed test tube to eliminate traces of methanol or water;
- dry in an oven at about 45°C under flow of inert gas, such as, nitrogen;
- add into the test tube 0.5 to 2 ml of the reagent as defined above and shake if necessary;
- heat the test tube to 100°C and maintain at this temperature for 1 hour, e.g. by means of a thermostatic bath or an oven;
- cool the test tube to room temperature, e.g., by immersing the tube into cold water or simply leaving it at room temperature for a suitable time span;
- take the liquid and pour into a test tube of the kind used for urine examination (if necessary, centrifuge the test tube to eliminate turbidity);
- insert the tube in a "first-level screening apparatus" for the quantitative determination of the substances sought for (benzoylecgonine, morphine, methadone, etc);
- adjust settings of the first-level apparatus in a way suitable for small amounts (this may be necessary if the apparatus is alternatively used for determination in hair or urine);
- perform screening –type analyses using the reagents provided with the kit normally employed for the examination of urine;
- read data resulting from the first-level analyses and establish positivity or negativity with reference to the cut-off value.

The above procedure may then be complemented with the following additional steps when confirmation analyses are required:

- arrange the samples in the order of increasing concentration of the sought for substances, determined as above described;
- perform confirmation analyses by analyzing the samples taken in the order of the arrangement (In this way the above mentioned problems of dragging traces of drugs from one sample to the other are overcome).

The invention process provides for the use of a reagent (hereinafter referred to as VMA) which is a buffer solution. The buffer serves for transforming the cocaine present in the hair into its metabolite benzoylecgonine, as shown in the scheme of Figure 14. Cocaine, in the presence of hydroxyl groups and at a suitable temperature first is transformed into an intermediate product and then into benzoylecgonine plus methanol.

Buffer solutions are obtained by reacting a salt with its weak base. These solutions have a stable pH; therefore the VMA reagent is able to produce hydroxyl groups in a steady way. The use of solutions in which the production of hydroxyl groups is not regular creates problems when the cocaine concentration is close to the cut-off limit.

As said above, the composition of the buffer for use in the process is preferably the following:

$$VMA = 0.2\ M\ (NH_4)_2HPO_4 + 5ml/L\ 25\%\ NH_4OH$$

Alternatively, VMA may be replaced by solution in which the component being the source of hydroxyl groups is selected among the following non-limitative list of substances: aluminum hydroxide, barium hydroxide octahydrate, benzyltriethylammonium hydroxide, benzyltrimethylammonium

hydroxide, calcium hydroxide, phenylhydrargirium hydroxide, lithium hydroxide, lithium hydroxide monohydrate, magnesium hydroxide, potassium hydroxide, potassium hydroxyantimoniate, sodium hydroxide, sodium hydroxide monohydrate, strontium hydroxyde octahydrate, tetramethylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide.

As solvent, any of the following can be used in alternative:
ethanol, methanol, water, monobasic ammonium phosphate, ammonium acetate, ammonium benzoate, ammonium bicarbonate, ammonium bichromate, ammonium bisulphate, ammonium bromide, ammonium carbamate, ammonium carbonate, ammonium citrate bibasic, ammonium chromate, ammonium iodide, molibdate, ammonium monovanadate, ammonium nitrate, ammonium oxalate monohydrate, ammonium persulphate, ammonium sulphate, ammonium sulphamate, ammonium sulphite, ammonium sulphide, ammonium tartrate, ammonium thiocyanate, ammonium thioglycolate, ammonium thiosulphate, ammonium chloride, sodium phosphate monobasic, sodium phosphate bibasic, potassium phosphate monobasic, potassium phosphate bibasic.

Figure 15 shows a graph in which the percentage of cocaine transformed into benzoylecgonine is reported versus the concentration of OH^- when the reaction is carried out at the temperature of 100°C for 1 hour. From the graph it appears evident that the transformation is maintained at high levels (at least 70%) for a range of OH^- concentrations of from 0.03 to 0.5 M.

From the graph of Figure 16, which shows hydroxyl concentrations after, respectively, 0, 15, 30 and 60 minutes in a reaction carried out at 150°C; it appears evident that the percentage of transformed cocaine is high only around the abscissa point of 15 minutes.

From all of the above and numerous other experiments the optimal values for the reaction temperature and time result to be, respectively, 100°C and 1 hour.

The examples reported below show the quantitative determination of cocaine in hair performed both with known techniques and with the process of the invention. Analyses have been carried out using the following instruments: for the screening analyses, the ROCHE instrumentation named "COBAS MIRA PLUS" which uses reagents provided by the same company and named "ABUSCREEN ON LINE"; for GC/MS the instrument provided by the company VARIAN which is named "SATURN GC/MS", model 4D.

EXAMPLES

Before proceeding to the various screening analyses the apparatus has been controlled with a sample positive to both cocaine and morphine in order to verify feasibility of the methodology.

As can be seen from Table 1 the sample resulted positive to both cocaine and morphine:

TABLE 1

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.16 ng/ml	POSITIVE
COCAINE	0.15 ng/ml	POSITIVE

The concentration values detected by the apparatus resulted to be 0.16 for morphine and 0.15 for cocaine, in line with the expected values for both substances present in the control sample.

EXAMPLE 1

In this example a sample has been analyzed which resulted, in the end, to be positive to both cocaine and morphine.

The sample was subjected to conventional analysis with acid hydrolysis and then subjected to screening analysis which gave the following results:

TABLE 2

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.13 ng/ml	POSITIVE
COCAINE	0.01 ng/ml	NEGATIVE

As can be seen, acid hydrolysis was able to extract an amount of morphine higher than the limit, but the amount of cocaine resulted to be too little to allow detection by the screening apparatus. The reason is that cocaine was extracted as such and not transformed into its metabolite benzoylecgonine, which latter is just what present screening methods detect.

The same sample was then subjected to confirmatory analysis by GC/MS with the results reported in Figures 1 to 4. Specifically, from Figure 1 it can be seen that the chromatogram of the first sample shows a high peak at the position of cocaine and a very small peak at the position of benzoylecgonine. The same Figure 1 also shows confirmation of the presence of morphine.

Additional confirmations of the presence of both cocaine and morphine result from the graphs of Figures 2, 3 and 4.

The sample of Example 1 was then subjected to the procedure of this invention to give the results shown in Table 3.

TABLE 3

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.23 ng/ml	POSITIVE
COCAINE	0.37 ng/ml	POSITIVE

It is immediately clear that the values detected for both morphine and cocaine are higher than the respective limits, therefore the sample is positive for both.

The high value detected for cocaine is to be ascribed to the benzoylecgonine that has been produced during the transformation reaction.

The same sample of Example 1 treated with the process of this invention has been subjected to GC/MS confirmation analyses with the results reported in Figures 5 to 7. As can be seen from Figure 5, the cocaine peak practically cannot be seen anymore because cocaine has been completely transformed in benzoylecgonine, the peak of which is well apparent in the same Figure 5.

EXAMPLE 2

Again in this example a sample which, in the end, resulted to be positive to both cocaine and morphine has been analyzed first by the conventional analytical method of acid hydrolysis and screening with the results reported in Table 4.

TABLE 4

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.32 ng/ml	POSITIVE
COCAINE	0.03 ng/ml	NEGATIVE

Like in the preceding Example, acid hydrolysis was able to extract an amount of morphine higher than the limit, but the quantity of cocaine extracted was too little to allow detection by the apparatus.

The sample was then subjected to GC/MS confirmatory analyses to give the results reported in Figure 8 where it can be seen that the chromatogram shows a high peak at the position of cocaine and a negligible peak at the position of benzoylecgonine.

The sample of Example 2 was then subjected to the invention process and to screening analyses with the results reported in Table 5.

TABLE 5

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.28 ng/ml	POSITIVE
COCAINE	0.99 ng/ml	POSITIVE

Both the cocaine and the morphine values are higher than the respective limits, therefore the sample is positive to both.

The sample of this Example, treated according to the invention process, was also subjected to GC/MS confirmation analyses with the results reported in Figure 9 where it can be seen that the cocaine peak is not practically present anymore whereas the benzoylecgonine peak is high.

EXAMPLE 3

In this Example a sample has been analyzed which, at the end, resulted to be positive to cocaine only and not to morphine.

This sample was first subjected to acid hydrolysis and screening by the conventional methods to give the results reported in Table 6.

TABLE 6

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.02 ng/ml	NEGATIVE
COCAINE	0.01 ng/ml	NEGATIVE

By acid hydrolysis a quantity of morphine lower than the limit was extracted. The quantity of cocaine was also too little to allow the equipment to detect it. By using the methods known in the art, therefore, nothing can be said as to the positivity or negativity of the sample and it was necessary to make recourse to the GC/MS confirmation analyses that gave the results reported in Figure 10. In such Figure, the chromatogram of the sample of this Example shows a high peak at the position of cocaine and a negligible peak at the position of benzoylecgonine. This confirms once more that acid hydrolysis extracted cocaine as such without any transformation.

The same sample of this Example has also been treated according to the invention process and subjected to screening analysis. Results are reported in Table 7.

TABLE 7

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.03 ng/ml	NEGATIVE
COCAINE	0.13 ng/ml	POSITIVE

It is immediately evident that while the value of morphine is lower than the limit, that of cocaine is slightly higher than the cut-off and therefore the sample is only positive to cocaine.

This same sample, after treatment with the invention process, has been subjected to GC/MS confirmation analyses with the results shown in Figure 11. In this Figure, the peak of cocaine has practically disappeared because the same was completely transformed into benzoylecgonine, the peak of which is, instead, very high and well apparent.

EXAMPLE 4

This fourth Example also analyzes a sample which, at the end, resulted positive to cocaine and not to morphine.

Analyses were carried out by applying the same scheme as in the preceding Examples.

Table 8 shows that upon analysis with acid hydrolysis the sample seems to be completely negative to both morphine and cocaine, whereas Figure 12 indicates the presence of cocaine in an amount higher than the limit.

TABLE 8

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.02 ng/ml	NEGATIVE
COCAINE	0.05 ng/ml	NEGATIVE

The same sample, treated with the invention process (VMA reagent) already on the first screening analysis shows a presence of cocaine higher than the limit, see Table 9.

TABLE 9

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.02 ng/ml	NEGATIVE
COCAINE	0.64 ng/ml	POSITIVE

As usual, this result was confirmed by GC/MS analysis as shown in Figure 13. The big peak of benzoylecgonine indicates that, before the treatment, the sample contained cocaine in a percentage higher than the cut-off.

The instant application is based upon Italian patent application VR99A000059, filed on 20 July 1999, the disclosure of which is hereby expressly incorporated by reference thereto, and the priority of which is hereby claimed.

AMENDED CLAIMS

1. A screening-type process for the quantitative determination of cocaine and other alkaloids which are present in a solid sample which includes the steps of:

a) preparing a solid sample in a finely divided or powdered form;

b) selecting a liquid reagent providing constant concentration of hydroxyl groups suitable for extracting and transforming cocaine into benzoylecgonine and for extracting other similar substances;

c) extracting cocaine and other similar substances contained in the sample and transforming the extracted cocaine into benzoylecgonine by maintaining the sample completely immersed in said liquid reagent at a temperature ranging from 10°C to 250°C for a period of time ranging from few seconds to 48 hours; and

d) analysing the liquid separated from the solid sample to determine the concentration of benzoylecgonine contained in said liquid with respect to the cut-off limit using a conventional screening kit for the determination of the said substance in urine.

2. Process according to claims 1, wherein said solid sample is a sample of hair.

3. Process according to claims 1 and 2, wherein said temperature is ranging from 100°C to 150°C.

4. Process according to claims 1 and 2, wherein said period of time is ranging from 15 minutes to 24 hours.

5. Process according to any preceding claims, wherein said temperature is maintained at 100°C for 1 hour.

AMENDED SHEET

6. Process according to claim 1, wherein said liquid reagent is an ammonia buffer comprising 0.2 M (NH4)2HPO4 with the addition of 5 ml of 25% NH4OH to each liter thereof.

7. Process according to claim 6, wherein the concentration of hydroxyl groups in said ammonia buffer is in the range of from 0.0001M to 5 M.

8. Process according to claim 6, wherein the concentration of hydroxyl groups in said ammonia buffer is in the range of 0.03M to 0.5 M.

9. Process according to claim 6, wherein the concentration of hydroxyl groups in said ammonia buffer is in the range of 0.04M to 0.33 M.

10. Process according to any preceding claims, wherein the analyzed samples are arranged in increasing order of concentration of cocaine or other alkaloids.

11. Process according to claim any preceding claims, wherein the samples are subjected to confirmation analyses with standard techniques such as GC or GC/MS.

12. Process according to any preceding claims, wherein each hair sample is made of about 50mg to 300 mg of finely divided and/or powdered hair.

13. Process according to claim 1, wherein said liquid reagent is a solution comprising a solute selected among aluminum hydroxide, barium hydroxide octahydrate, benzyltriethylammonium hydroxide, benzyltrimethylammonium hydroxide, calcium hydroxide, phenylhydrargirium hydroxide, lithium hydroxide, lithium hydroxide monohydrate, magnesium hydroxide, potassium hydroxide,potassium hydroxyantimoniate, sodium hydroxide, sodium hydroxide monohydrate, strontium hydroxyde octahydrate,

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tetramethylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide, dissolved in a solvent selected among ethanol, methanol, water, monobasic ammonium phosphate, ammonium acetate, ammonium benzoate, ammonium bicarbonate, ammonium bichromate, ammonium bisulphate, ammonium bromide, ammonium carbamate, ammonium carbonate, ammonium citrate bibasic, ammonium chromate, ammonium iodide, molibdate, ammonium monovanadate, ammonium nitrate, ammonium oxalate monohydrate, ammonium persulphate, ammonium sulphate, ammonium sulphamate, ammonium sulphite, ammonium sulphide, ammonium tartrate, ammonium thiocyanate, ammonium thioglycolate, ammonium thiosulphate, ammonium chloride, sodium phosphate monobasic, sodium phosphate bibasic, potassium phosphate monobasic, potassium phosphate bibasic.

14. Diagnostic kit for the carrying out of the process according to any claims 1 to 13, comprising a liquid reagent with constant concentration of hydroxyl groups suitable for extracting cocaine and other alkaloids and transforming cocaine into benzoylecgonine, and a conventional screening kit for the determination of said metabolite in urine samples.

AMENDED SHEET

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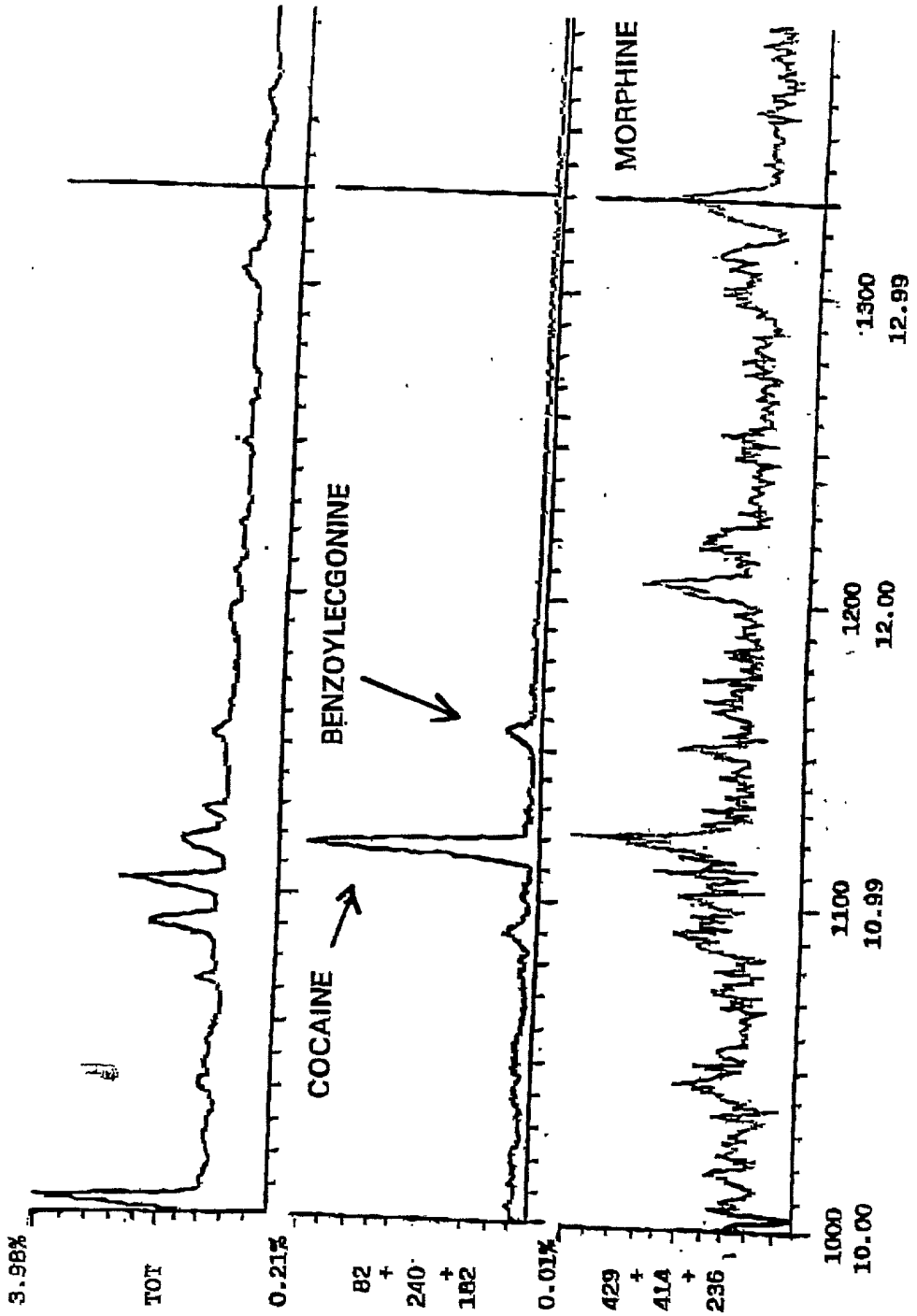
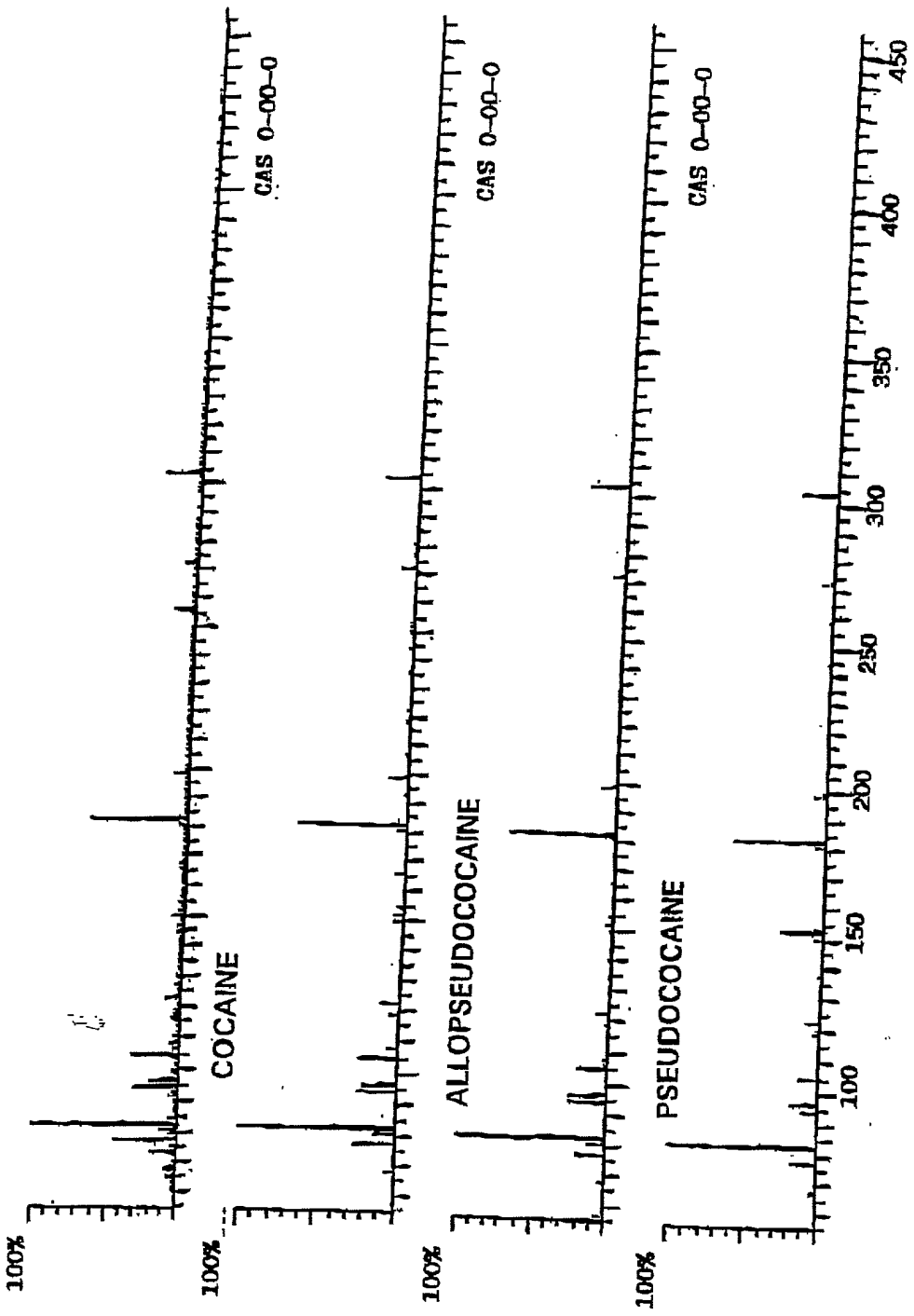


Fig. 1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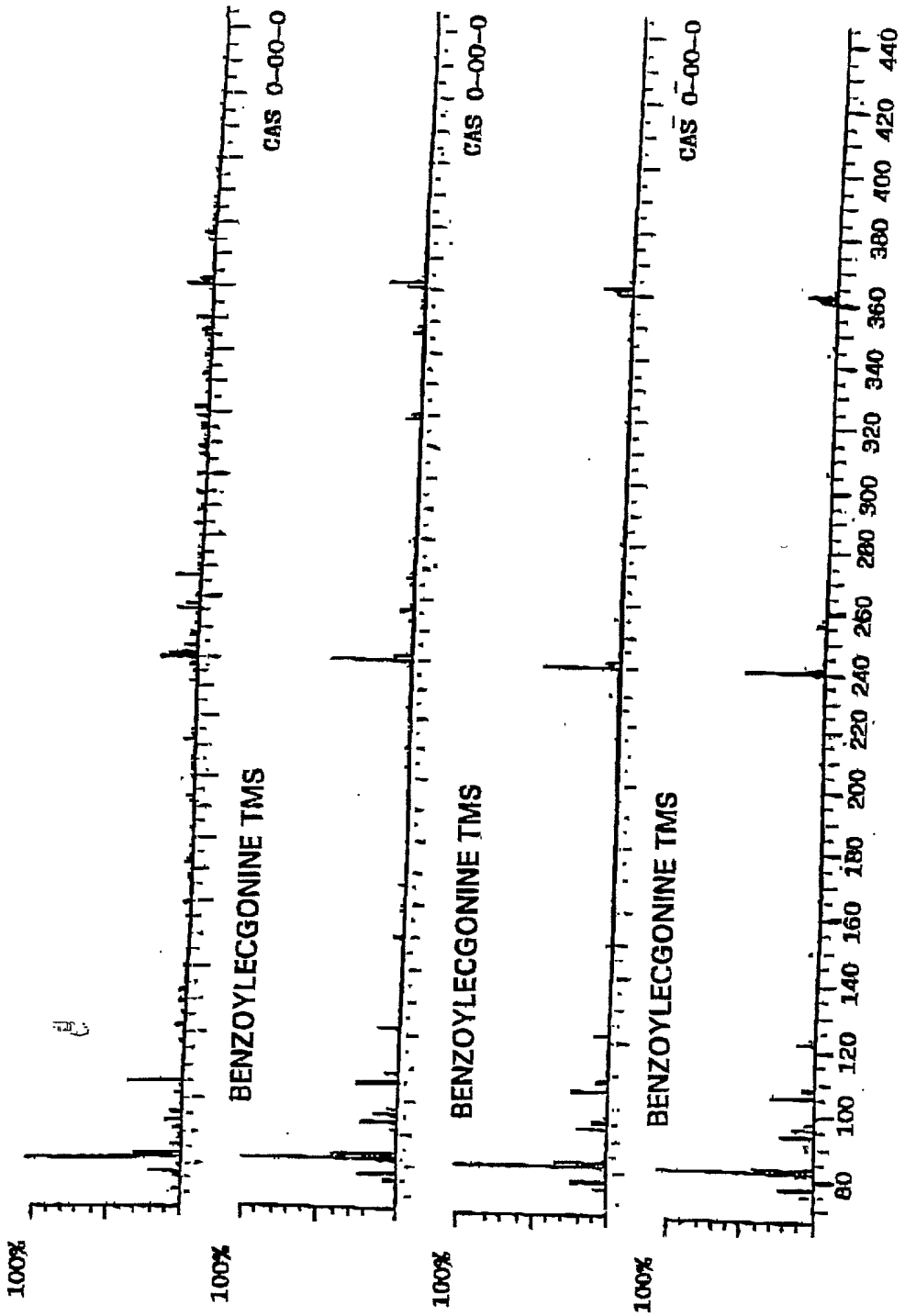
ALLOPSEUDOCOCAINE
CAS 0-00-0
PSEUDOCOCAINE
CAS 0-00-0
100%
100%
100%
100%
100
150
200
250
300
350
400
450

Fig. 2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CAS 0-00-0
100%
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440

Fig. 3

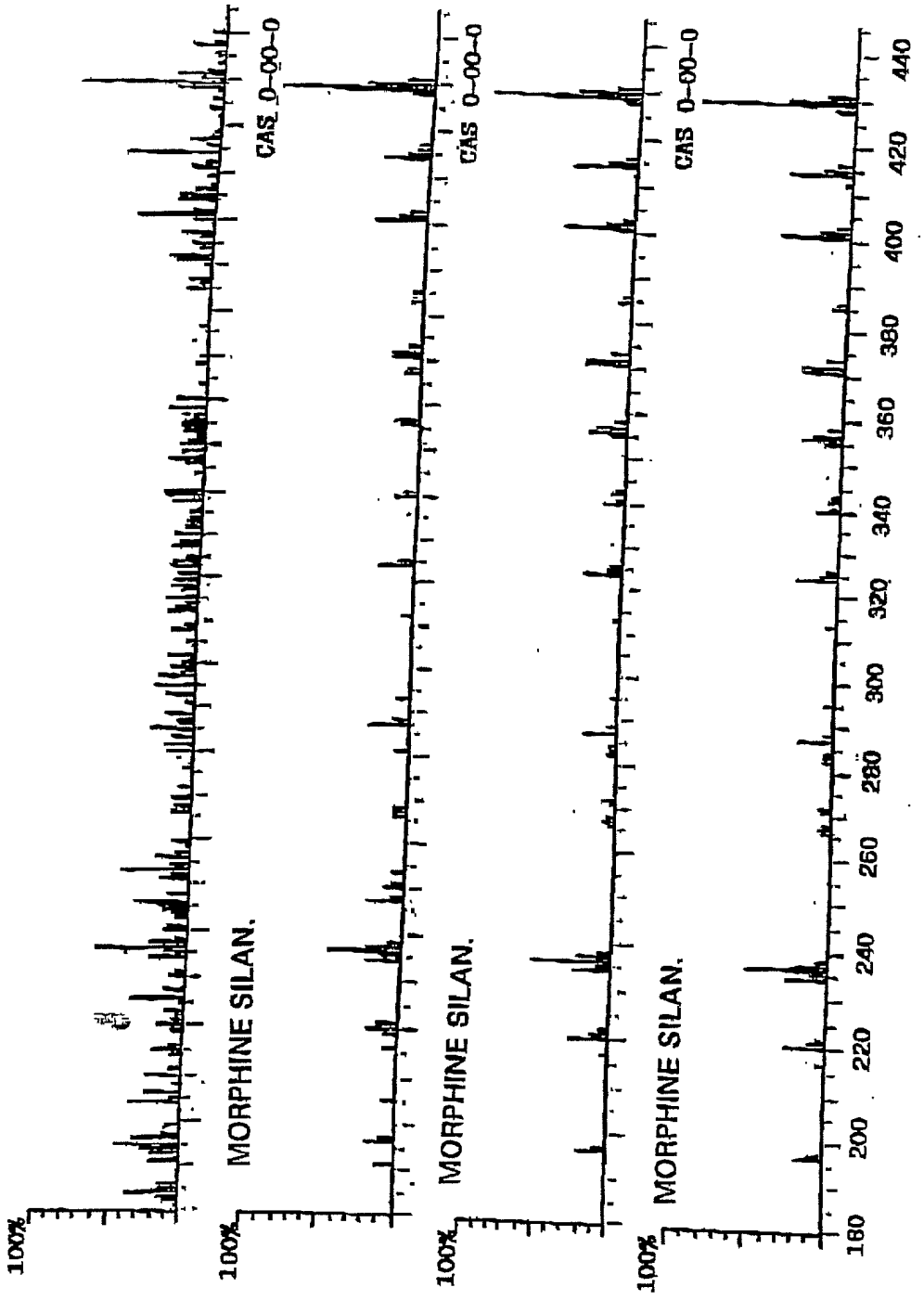
MORPHINE SILAN.
CAS 0-00-0
100%
180
200
220
240
260
280
300
320
340
360
380
400
420
440

Fig. 4

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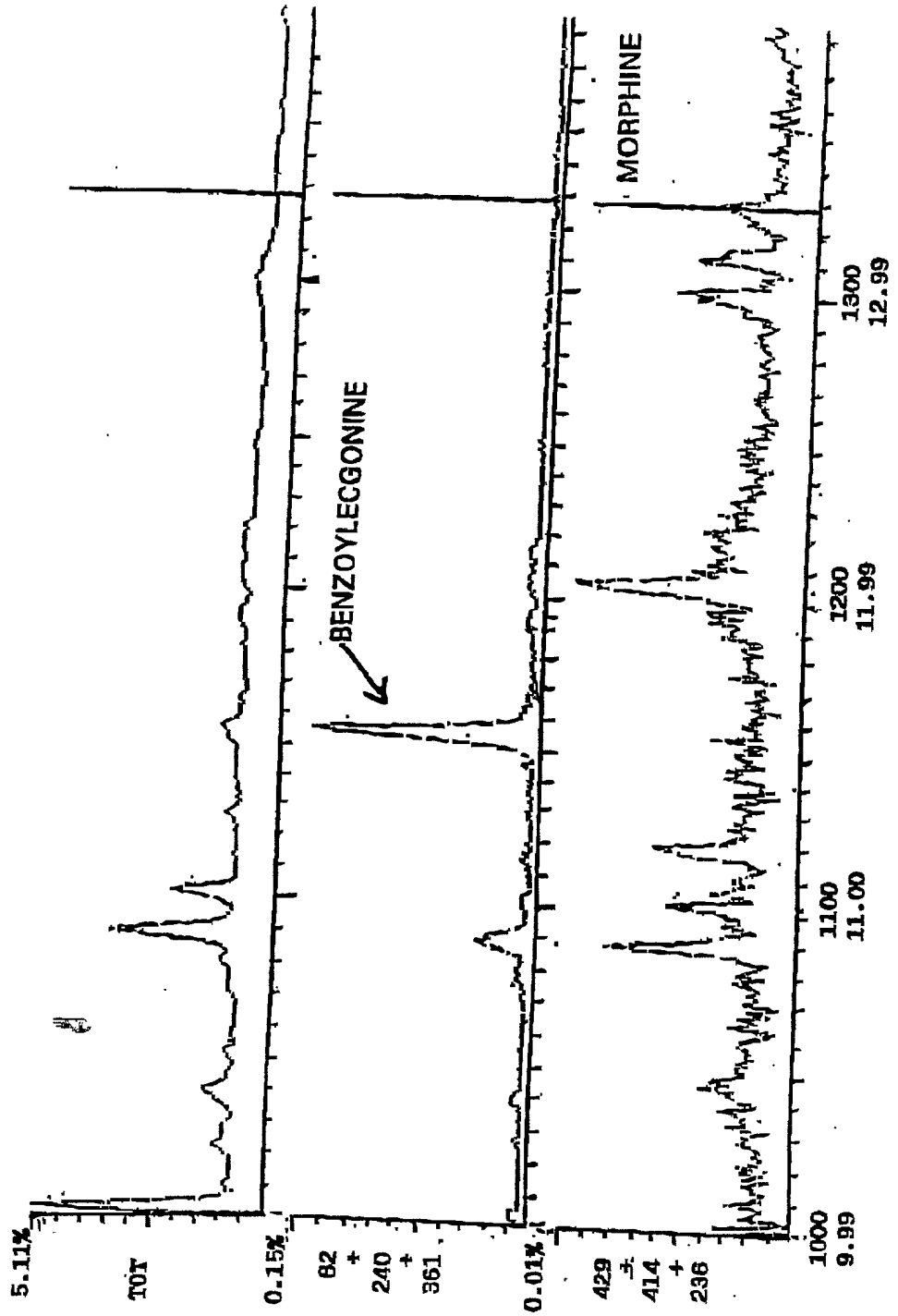

Fig. 5

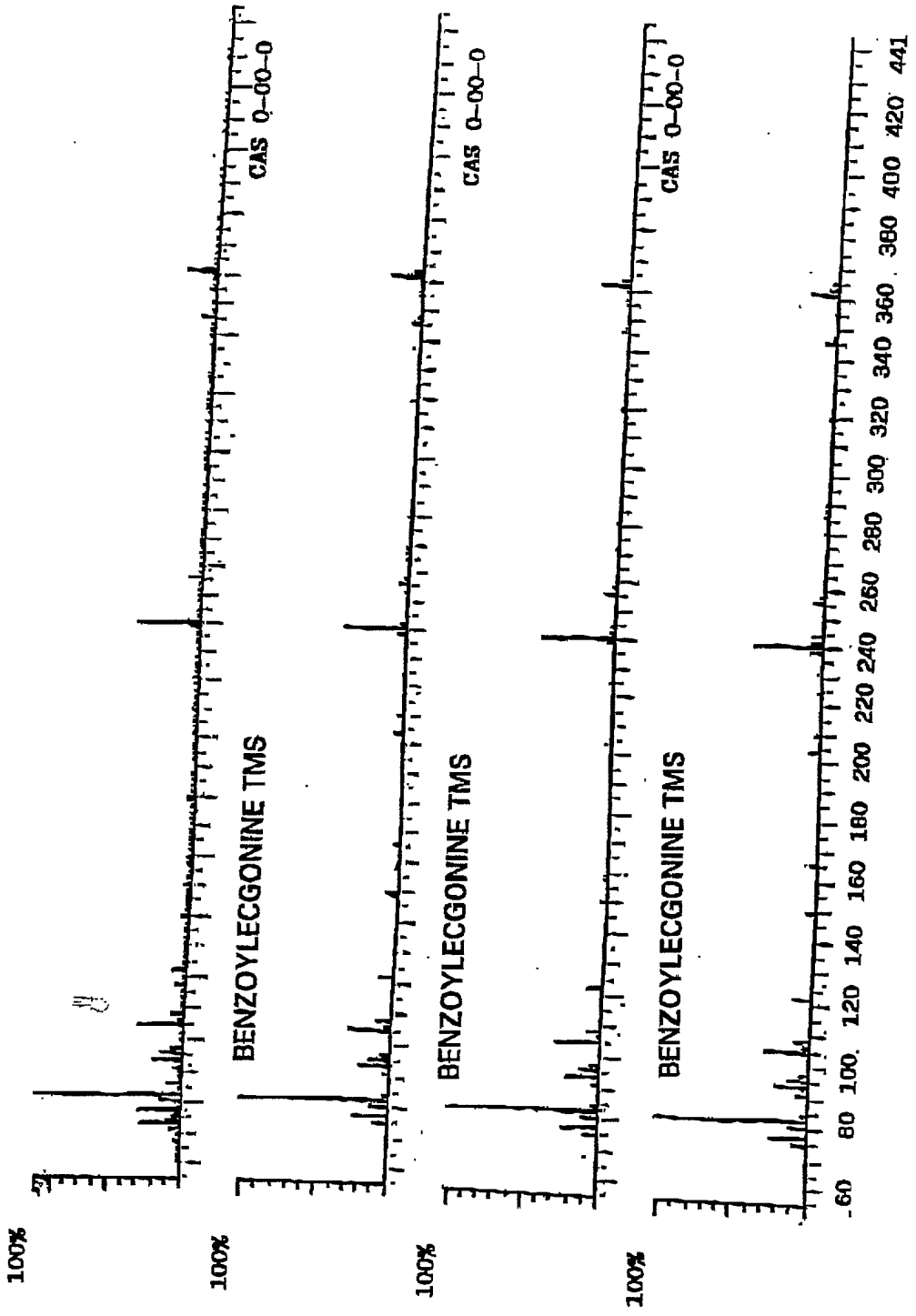

Fig. 6

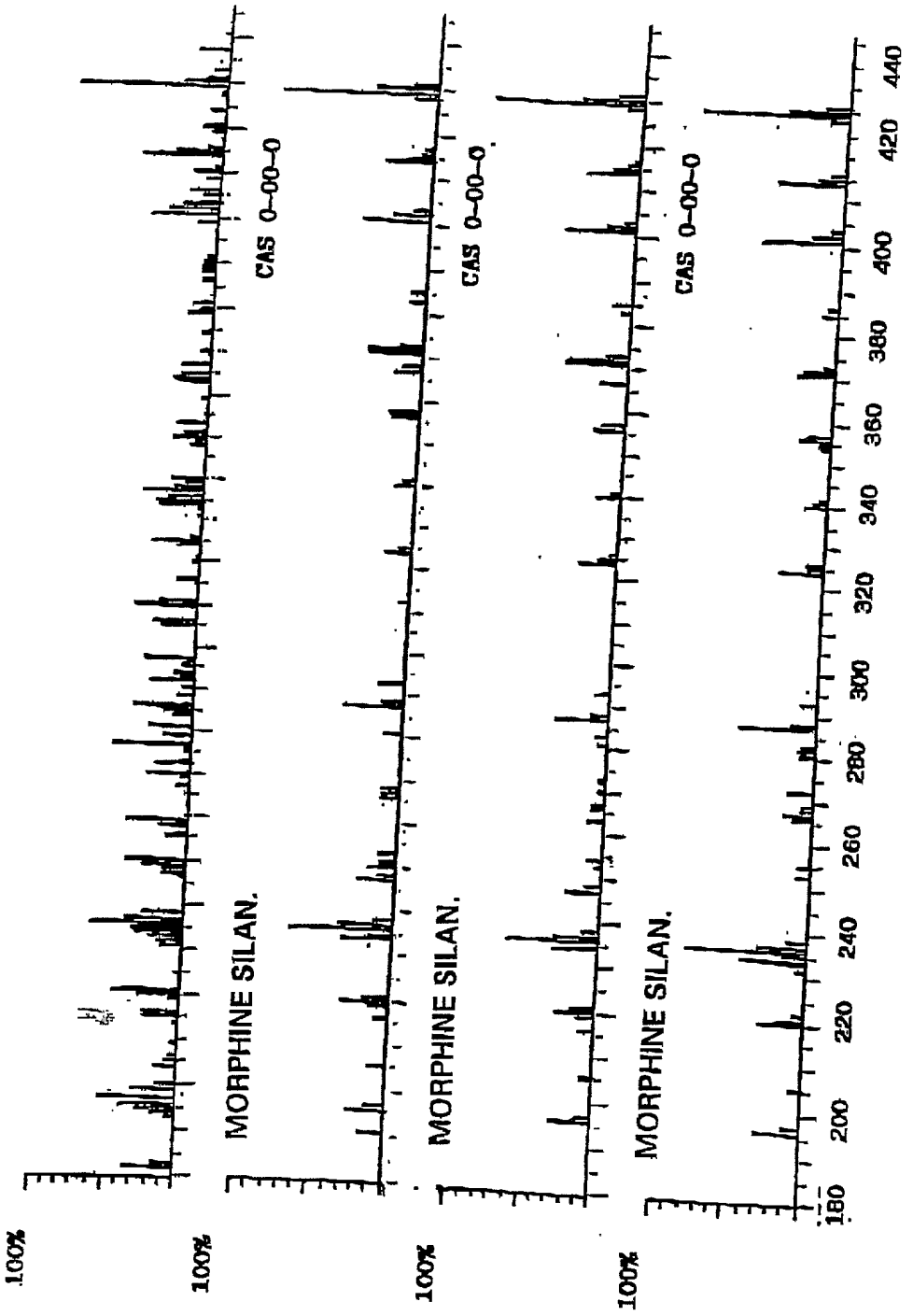

Fig. 7

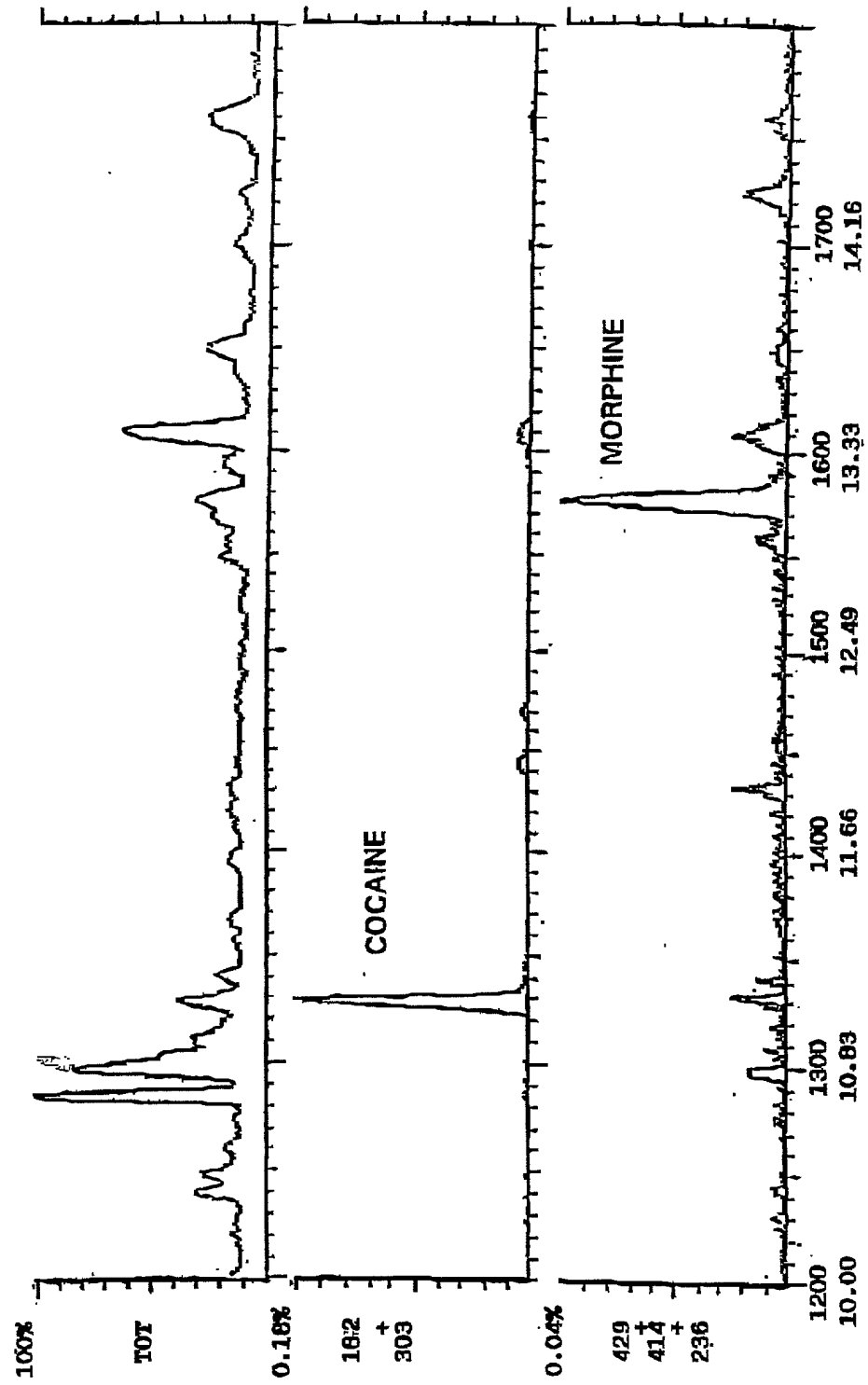

Fig. 8

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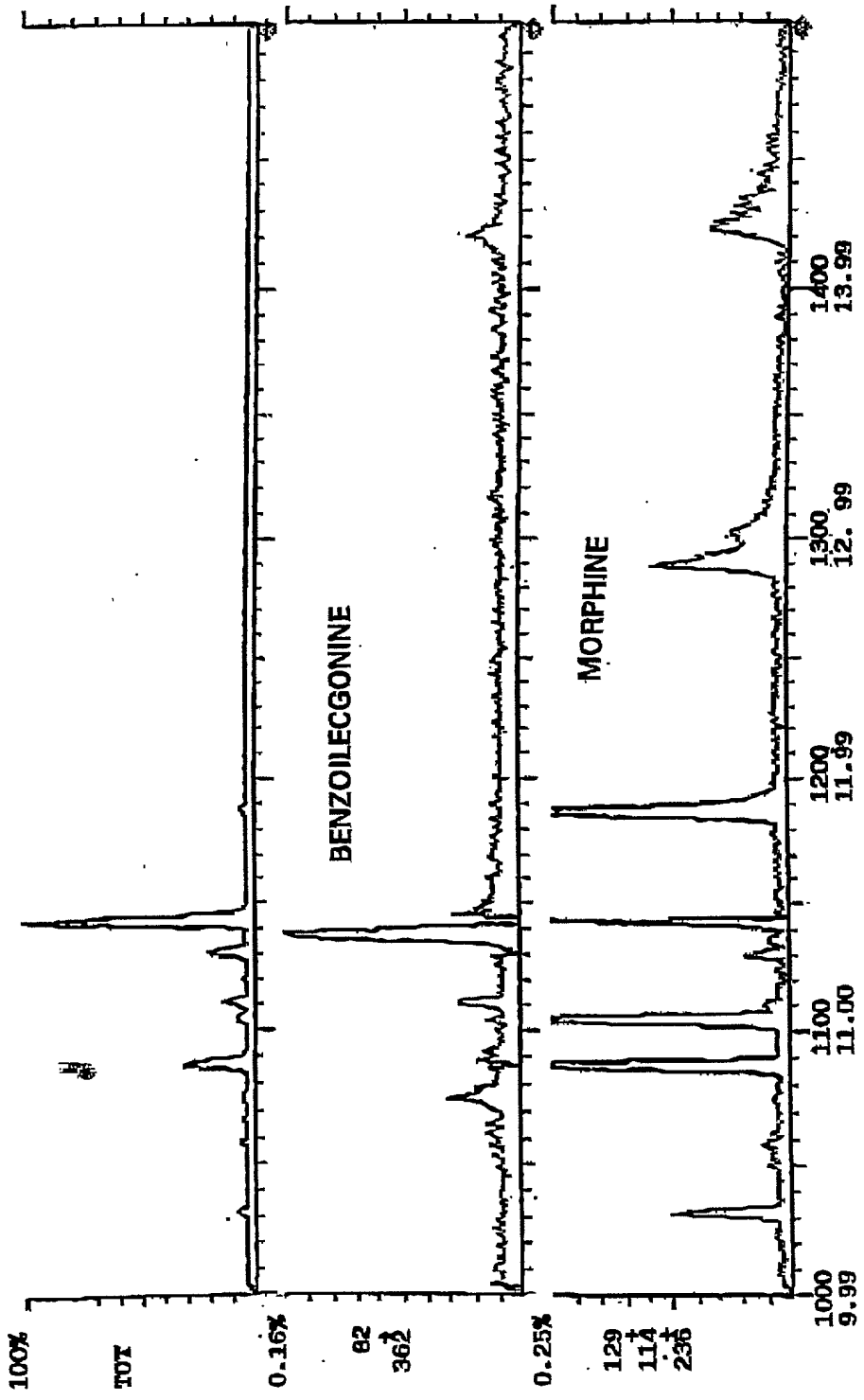

Fig. 9

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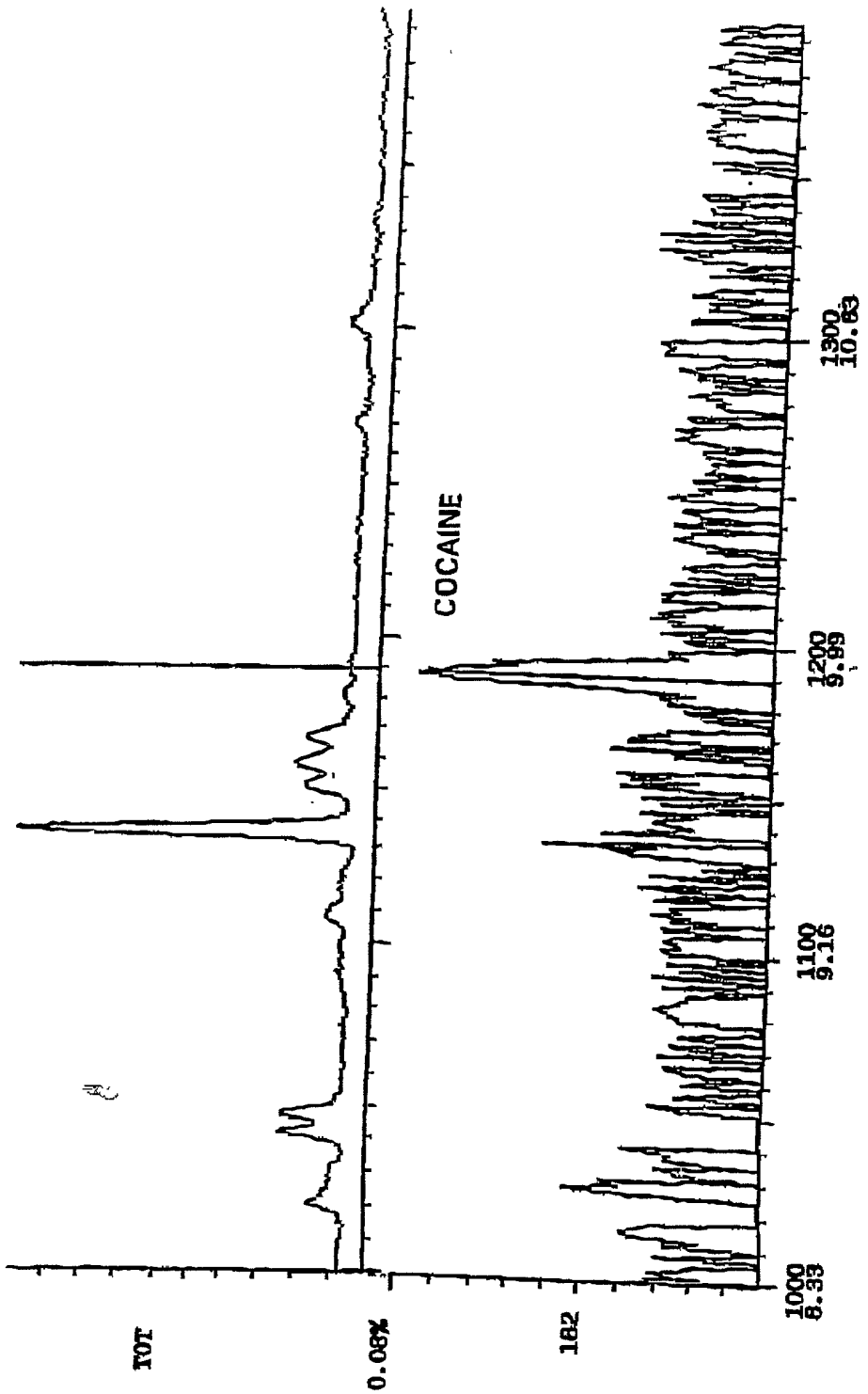

Fig. 10

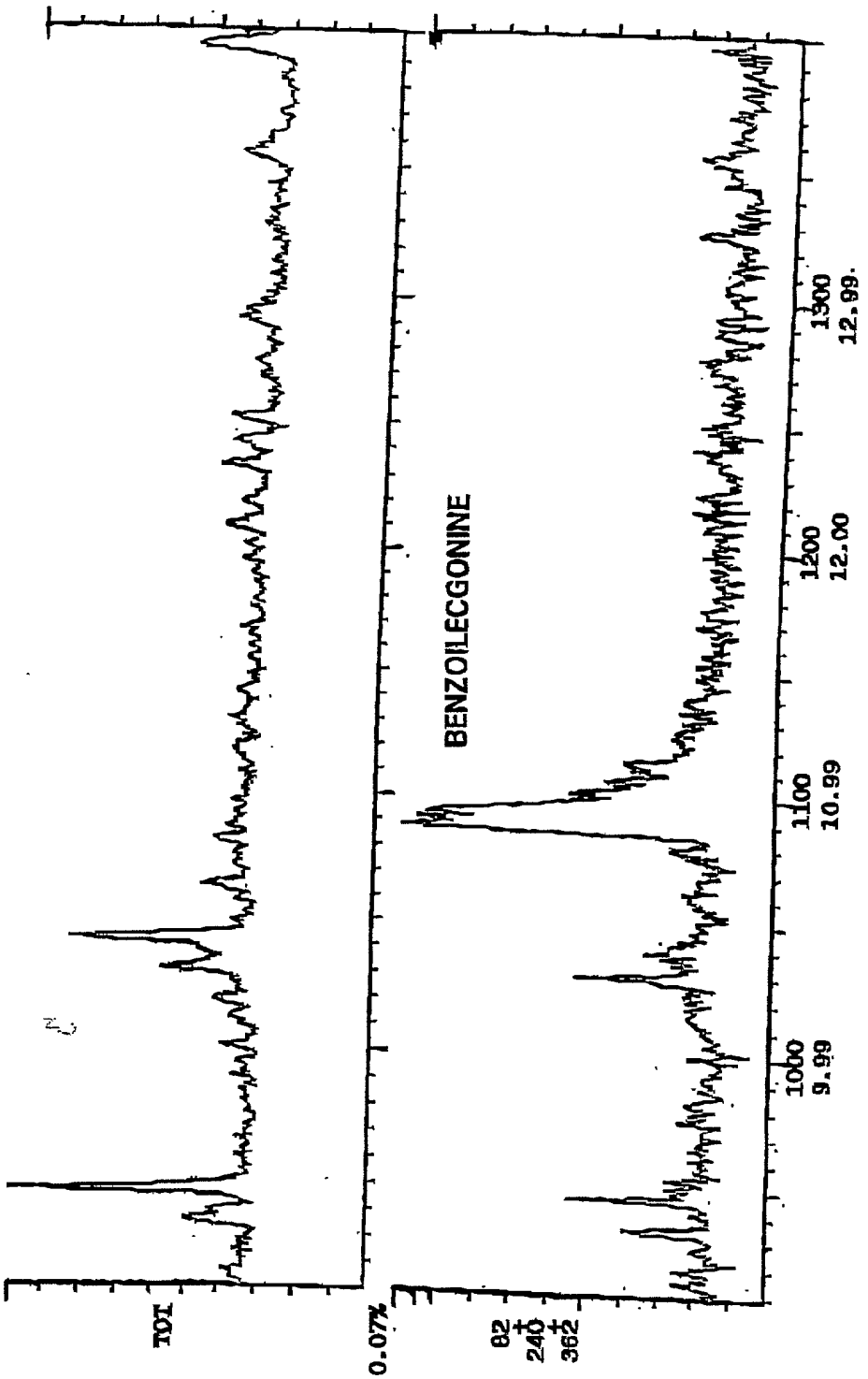

Fig. 11

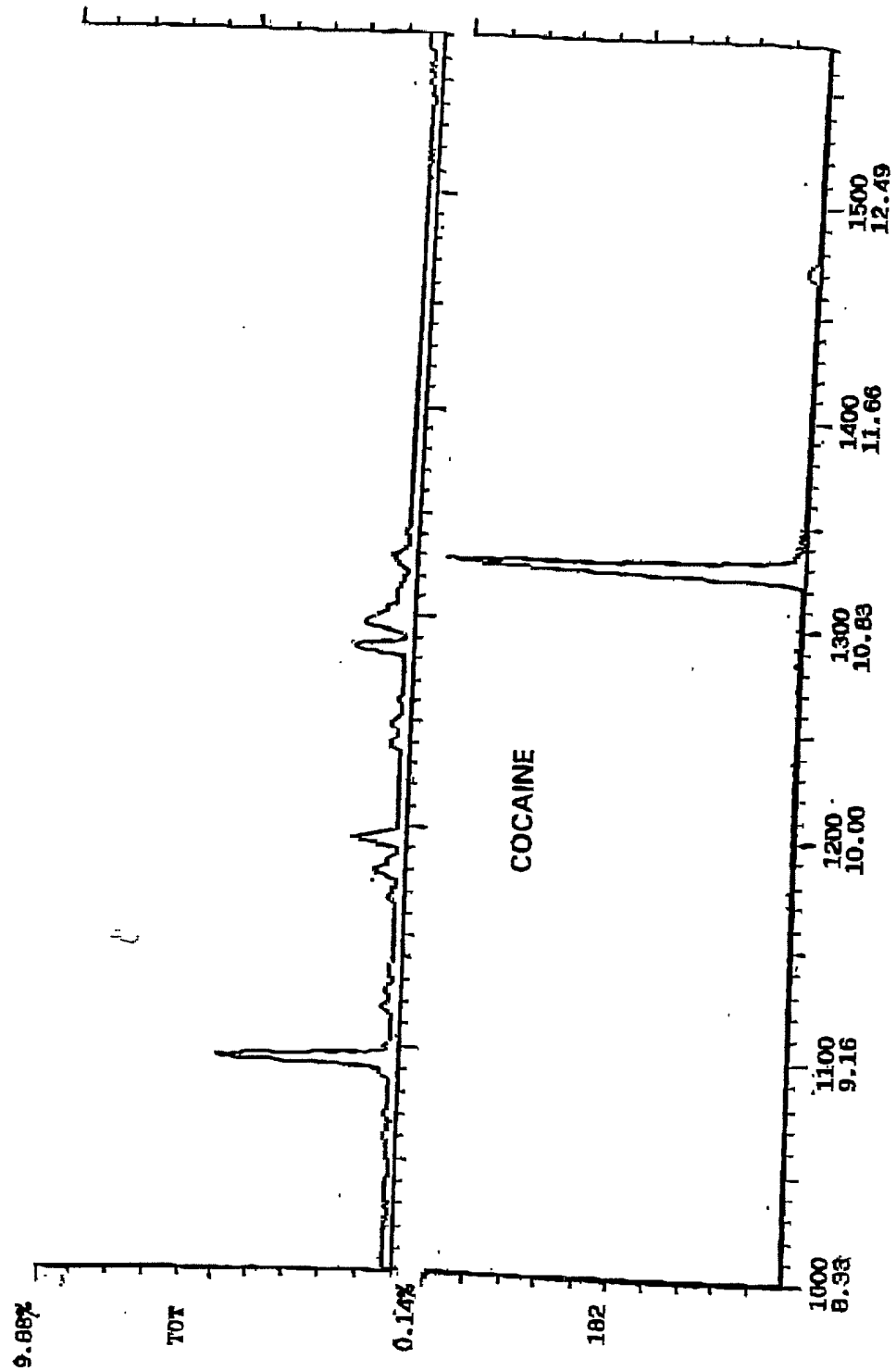

Fig. 12

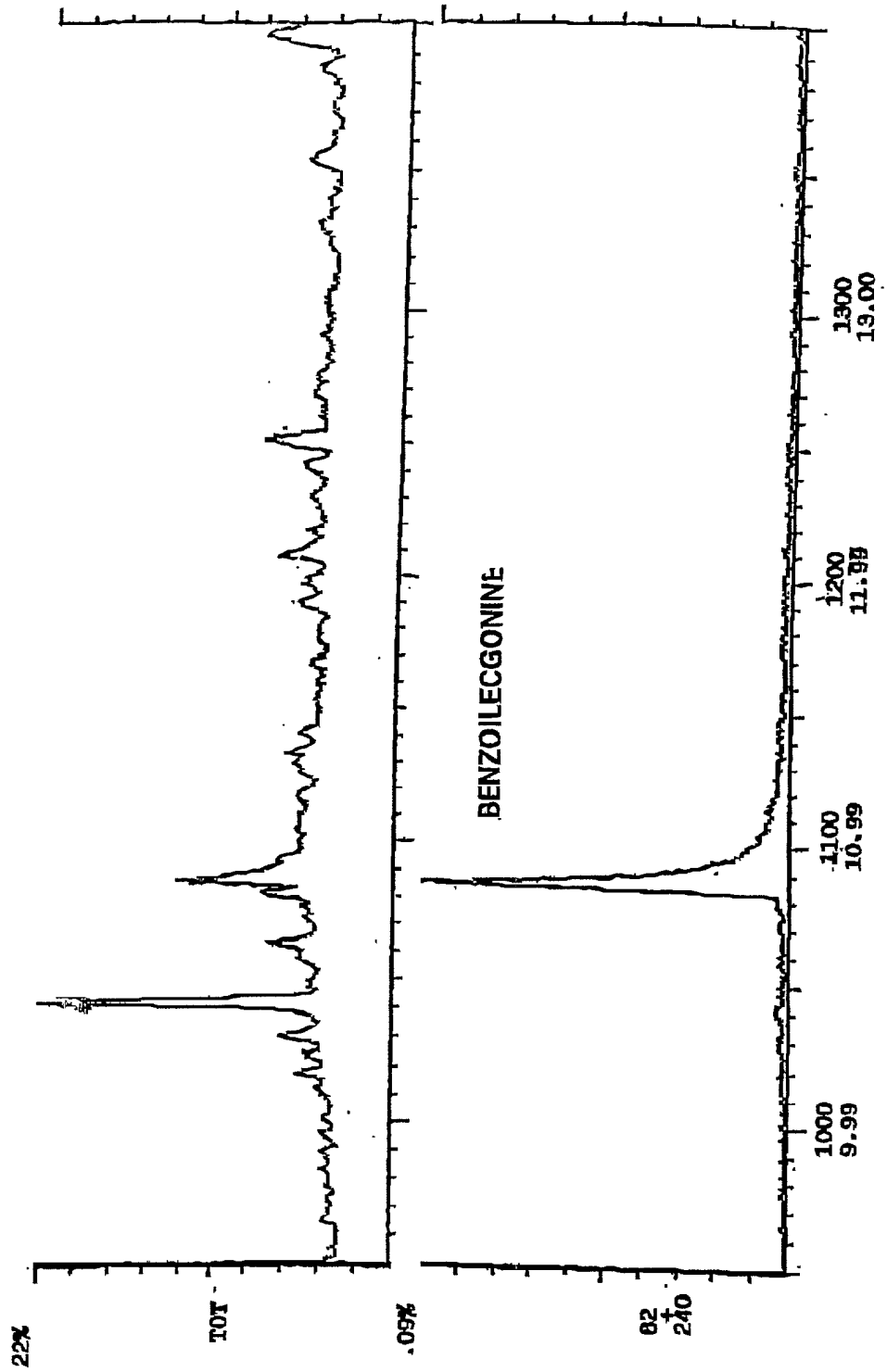

Fig. 13

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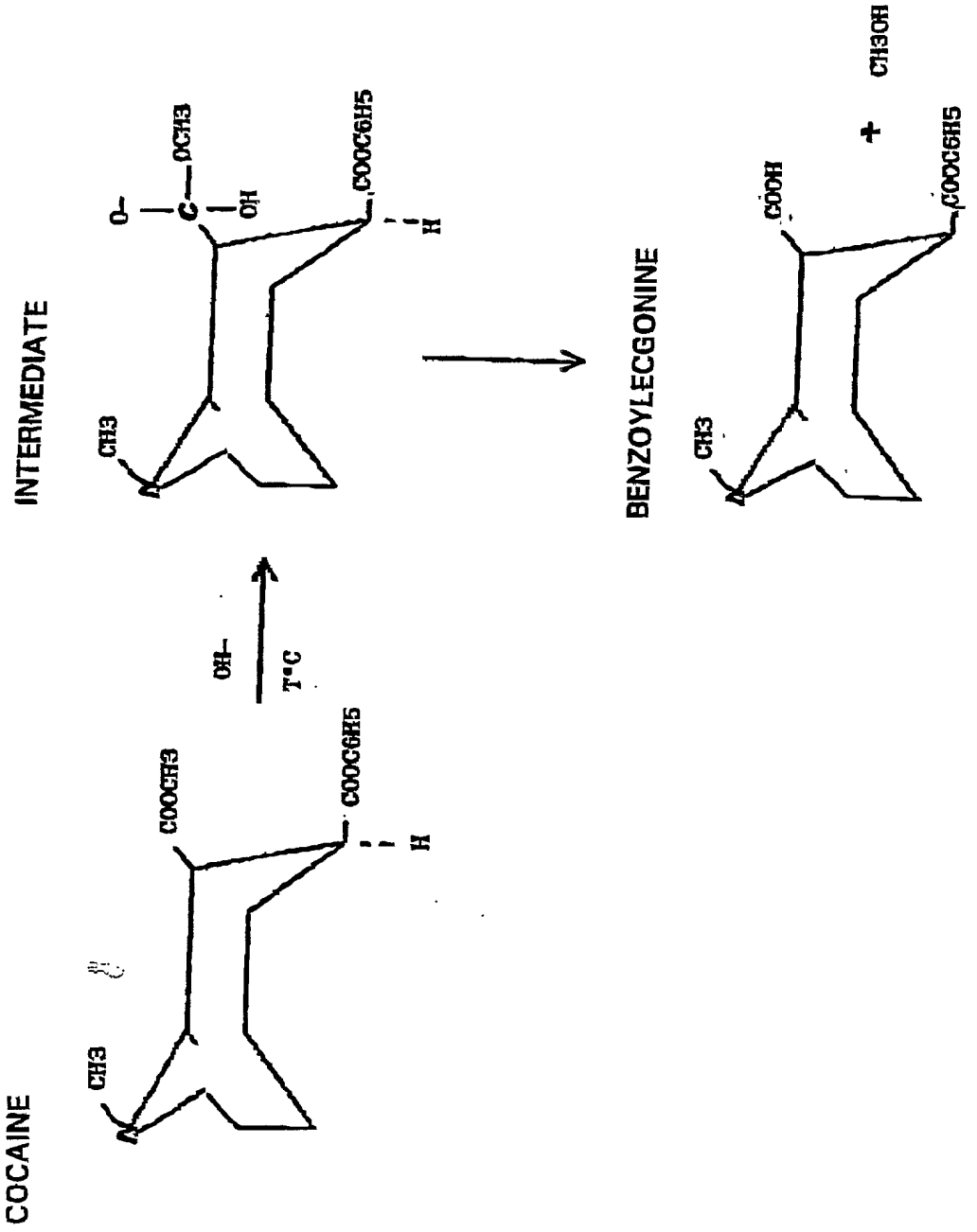

Fig. 14

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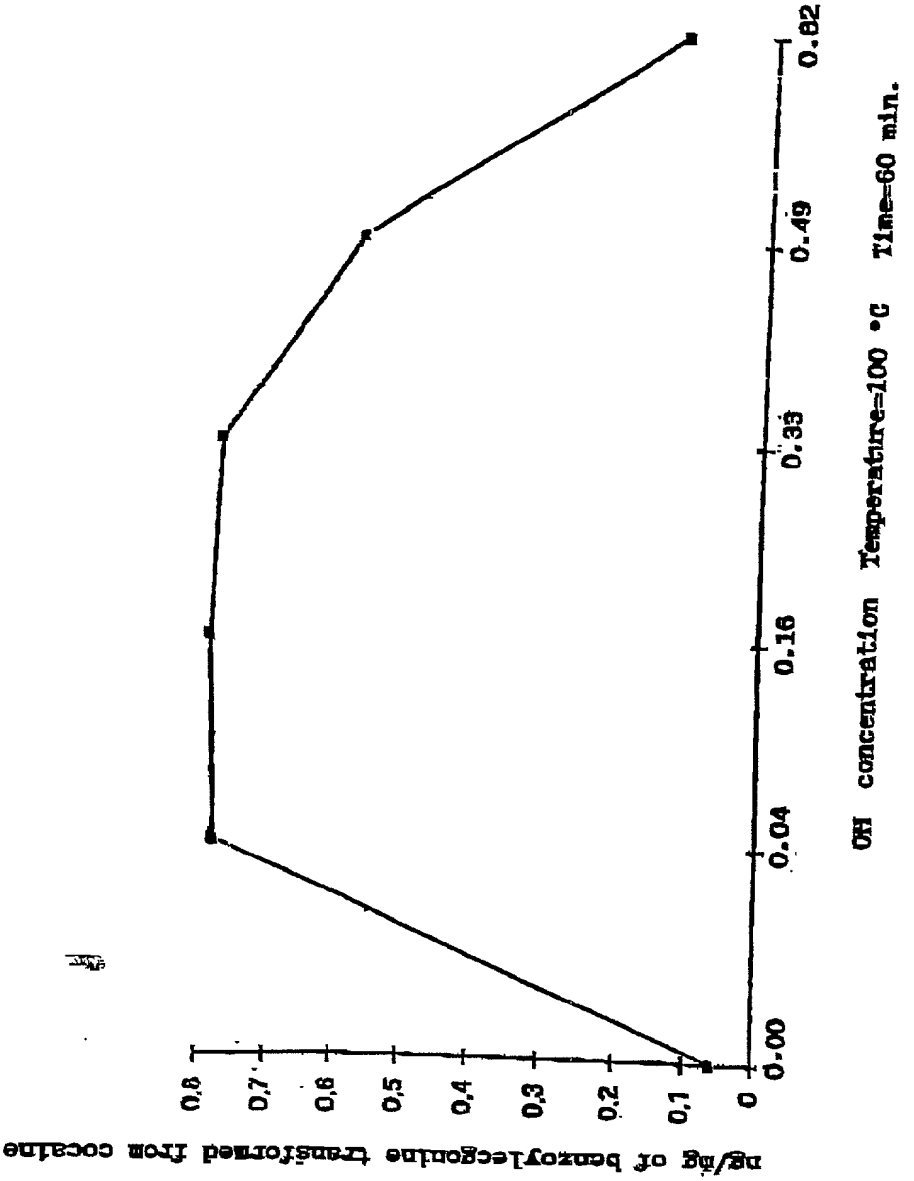

Fig. 15

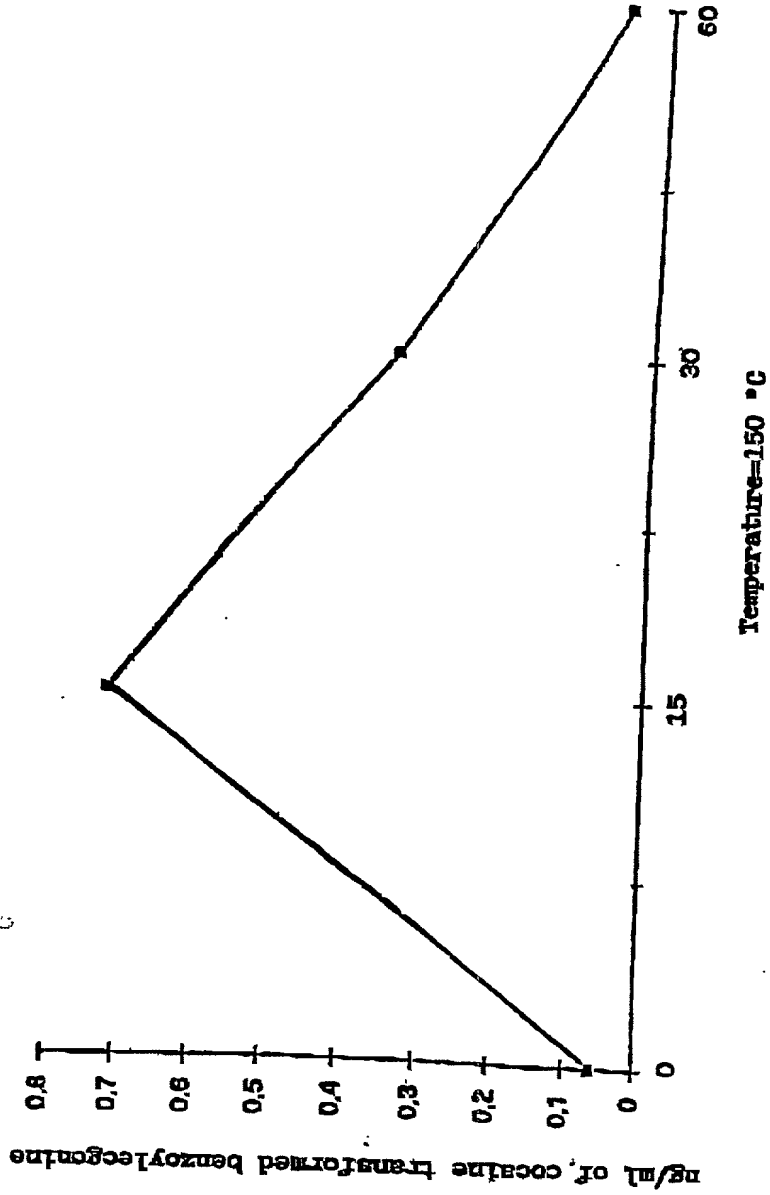

Fig. 16

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PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS.

This invention relates to a process for the quantitative determination of cocaine and other alkaloids, such as morphine and methadone, in a solid sample, e.g., a sample of hair, by using a screening type approach.

The invention also relates to a reagent for use in such process and new diagnostic kits including such reagent among their components.

By "screening type approach" is meant a kind of analysis permitting to analyze in a relatively short time span a relatively large number of samples in a cheap, efficacious and standardized manner. This kind of analysis permits to exclude the negative samples by immediately identifying the samples that do not contain the substance or the entire substance class or those in which said substances are present at a level lower than the threshold or cut-off value.

The threshold or cut-off is a practical limit selected to establish if the sample analyzed is positive or negative. The threshold value differs from the limit of detection of the method, that is, the lowest concentration of an analyte that can be determined. In fact, the cut-off value is normally set at a concentration higher than the limit of detection in order to obviate the imprecision of the analysis at values close to the limit of detection.

Cut-off values are conventionally established and take into account a multiplicity of factors, such as the capability of using commercially available reagents, the pharmacokinetic properties of the substances and the need to avoid false negatives (see: P.Zuccaro, S.Pichini, I.Altieri and R.Pacifici: Proposta di linee guida per l'analisi delle sostanze d'abuso nei liquidi biologici

in RAPPORTI ISTISAN 96/29 edited by ISTITUTO SUPERIORE DI SANITA').

The substances that the metabolism of the human body accumulates in the hair are numerous. Usually, it is required to detect the presence of alkaloids and other substances of abuse, such as, morphine, methadone and/or cocaine. By the method of this invention further substances can also be detected, such as, those of the following non-limitative list: 6-O- mono-acetyl-morphine, bi-acetyl-morphine, codeine, papaverine, nalorphine, nicotine, cotinine, caffeine, noscarpine, mepivacaine, trimetropin, buprenorphine, pentazocyne, methadone metabolyte, benzoylecgonine, amphetamine, metamphetamine, methylenedioxyamphetamine, methylenedioxymetamphetamine, methylenedioxyethylamphetamine, benzodiazepines, barbiturates.

Description of the state of the art

Several techniques are known for the determination of the analytes of interest, such as those mentioned above. Specifically, for the analysis of cocaine that is present in hair, gas chromatography (GC) combined with Mass Spectrometry (MS) hereinafter referred to as GC/MS and Radio Immune Assay technique (R.I.A.) are known.

GC or liquid chromatography (LC) combined with MS are methods of resolution, purification or separation and identification of components of complex mixtures of organic or inorganic substances having even strictly similar chemical properties. The separation of substances dissolved in a liquid or fixed on a finely divided or porous solid substance is based on percolation, respectively elution trough them of an eluent gas, respectively liquid.

When the substances to separate/detect can be made gaseous and as eluent a

gas is used, GC applies. The latter is a separation method based on the distribution between a solid or liquid stationary phase and a mobile phase made of the gases or vapors to separate, which are carried by a stream of an inert gas.

The analytical results are reported in a graph named chromatogram, in which the quantities of the single components present in the mixture and transferred to the eluent gas/liquid are reported versus time. The graph has peaks whose highness is a direct function of the quantity of the specific substance.

Chromatographic methods that can be used for confirmatory analyses are GC and LC, the latter being often referred to as High Performance Liquid Chromatography (HPLC). The most commonly employed detectors for GC are electron scatting detectors and phosphor nitrogen detectors.

As said above, GC/MS employs a gas chromatograph coupled to a mass spectrograph. By so doing, the separation capability of GC combines with the specificity proper of MS. Therefore, GC/MS represents the method of choice for confirmatory analyses of the above named substances as well as their metabolites (see again "Proposta di linee guida per l'analisi delle sostanze d'abuso nei liquidi biologici" by ISTITUTO SUPERIORE DI SANITA')

In the conventional acid hydrolysis, however, cocaine is extracted by hydrochloric acid as it is, i.e. without undergoing transformation into its metabolite benzoylecgonine. Since the amounts of cocaine extracted in this way are very little, it is not possible to determine cocaine by the usual screening methods.

Radio Immune Assay (RIA) is based on radio-immunological tests using known amounts of antibodies and of analyte labeled with a radioisotope (generally

I^{125}). During incubation the labeled analyte and that possibly present in the sample compete for the antibody sites. After precipitation of antigen-antibody complexes and centrifugation the supernatant or the precipitate are transferred to a gamma Geiger counter that measures the radioactivity level. RIA kits are extremely sensitive and allow identification of 1-5 ng of substance per ml. Adoption of automatic instruments for pipetting and counting allows the contemporaneous analysis of numerous samples with response times of 1 to 5 hours. On the other hand, the use of radioactive isotopes requires adequate safety measures. Furthermore, the relatively short half-life of the radioactive isotopes imposes a careful handling of reagents.

RIA does not lend itself to a widespread use for the quick determination of the substances in question in view, first of all, of the high cost of reagents, further increased by liability of the antisera. Secondly, in view of the criticality of the analysis due to the need of operating with radioactive materials that are dangerous for the analysts and also in view of the required times which are relatively long. RIA also requires the availability of rooms properly equipped and shielded, as well as particular care in handling waste materials and in their disposal.

Therefore, RIA is scarcely employed in this sort of analyses. The prior art shows therefore the impracticality of determining cocaine present in the hair by the so-called screening type approach, as described above, i.e., by very quick and cheap techniques as are used for the determination of the same substances in urine. This is mainly due to the fact that in the conventional screening-type approach what is analyzed is not cocaine, but its metabolite benzoylecgonine. The fact that this substance does not exist in nature by itself, but only as the metabolyte of cocaine and the fact that the reaction of transformation of cocaine into benzoylecgonine, i.e. the transformation of the ester group into an hydroxyl group, is irreversible in an alkaline environment

render the determination of benzoylecgonine a necessary and sufficient condition for the detection of cocaine.

Benzoylecgonine is found in the hair in a ratio of 1 to 4 or even 1 to 10 and more with respect to cocaine. Therefore, when the amount of cocaine is very low or close to the cut-off values (0.2-0.1 ng cocaine per mg hair) screening cannot be performed because of the low amount of this metabolyte (0.05-0.025 ng benzoylecgonine per mg hair), such amount being undetectable by the screening apparatuses available in the laboratories.

Summary of the invention

The main object of this invention is to overcome the above mentioned drawbacks, i.e. the impossibility to dose the cocaine present in a solid sample by the conventional screening-type approach.

In accordance with the invention, this object is achieved by a screening-type procedure for the quantitative determination of cocaine and other alkaloids which are present in a solid sample which includes the following steps:

- providing a solid sample in a finely divided and/or powdered form;
- completely immersing the sample into a liquid reagent providing a concentration of hydroxyl groups in the range of from 0.0001 to 5 M;
- maintaining the sample immersed in the liquid at a temperature of 10 to 250°C for a period of time from a few seconds to 48 hours; and
- analyzing the liquid separated from the solid with a conventional kit for the determination of the said substances in urine.

Preferred aspects of the invention include using hair as the solid material forming the solid sample; using a buffer as the reagent which provides hydroxyl groups, more preferably an ammonia buffer, most preferably a buffer

that is hereinafter referred to as VMA; and heating the sample immersed in the liquid at a temperature of about 100 to 150°C for about one hour.

In accordance with another aspect of the invention a process is provided which includes the additional steps of:

- arranging samples by increasing concentrations of the substances of interest; and
- performing confirmation analyses with known techniques, such as, GC or GC/MS.

In one embodiment of the invention, the process may provide the following steps:

- providing a sample made of about 50 to 300 mg of finely divided and/or powdered material;
- adding in the test tube containing the said sample a suitable liquid reagent until the sample is completely immersed, said reagent being capable of performing extraction and transformation of cocaine into benzoylecgonine and at the same time of extracting other similar substances which are present in the sample
- if necessary, agitating the test tube to facilitate immersion of the sample;
- heating the contents of the test tube to a temperature T1 for a time interval t1 by keeping the test tube immersed in a thermostated bath or by placing it in an oven;
- cooling the test tube to room temperature;
- taking the liquid and transferring it into a test tube suitable for a screening type instrument;
- performing the screening by using a kit of reagents for the determination of the said substances in urine;
- reading the data resulting from the first level instrumentation to verify the concentration values with respect to the cut-off limit; and

- contemporaneously determining the amount(s) of substance(s) present.

In accordance with another aspect of the invention a reagent is provided for use in the above mentioned process which in the most preferred embodiment has the following formula:

$$VMA = 0.2\ M\ (NH_4)_2HPO_4 + 5ml/L\ 25\%\ NH_4OH$$

In the above formula $(NH_4)_2HPO_4$ is dibasic ammonium phosphate and NH_4OH is ammonium hydrate. In fact, 5ml/L NH_4OH give a 0.07 M concentration of hydroxyl groups, which is comprised in the range giving 100% conversion.

In addition to the main advantage of the invention as outlined above, another advantage is that the confirmation analyses (e.g., GC/MS) need to be performed only on those samples that have been determined to be positive at the initial screening whereas, when conventional techniques are used, all samples must be analyzed since there is no kit available for determination of cocaine, but only for determination of benzoylecgonine.

In average, the analysis of 25 samples requires two hours for the preparation of the samples plus one week for the confirmation analyses of all (both positive and negative) samples.

On the other hand, by applying the present invention, an analyst will require for the same 25 samples two hours for samples preparation plus 30 minutes for the screening-type analysis plus the time necessary for performing the confirmation analyses. However, the latter need to be performed only on those samples which have been determined to be positive.

Since, in the average, positive samples are between 0 and 20% and since

most of the time required is spent in the confirmation analyses, the procedure according to the present invention allows saving of substantial time which is estimated at about 70 to 90% of the time required for the analysis of the 25 samples taken into consideration.

Another advantage according to this invention is that the possible dragging of the active substances from one sample to another in the confirmation analyses is eliminated or minimized because it is possible to arrange samples in order of increasing concentration of the substances of interest, therefore proceeding to confirmation analyses starting from those samples having the lowest concentration and then with those having higher and higher concentrations.

In this way, it is completely eliminated the possibility that a sample with high concentration of, e.g. cocaine, leaves a trace of it in the instrument used for the analysis and has an impact on the determination of the same substance in the following sample, perhaps having a concentration just below the cut-off.

Still another advantage of this invention is that it allows to search and determine at the same time and in the same sample not only cocaine but also other substances, such as, morphine by applying the same cut-off limit or else methadone by modifying the limit of cut-off.

One further advantage is that the procedure according to this invention offers a higher guarantee to the analyst because positive outcome is confirmed by two different analytical methods.

In a further aspect of the invention a diagnostic kit is provided including the reagent described above as one of its components.

Still further additional advantages will appear from the reading of the following detailed description and the following non-limitative examples.

Brief Description of the Drawings

Figure 1 is a chromatogram obtained from an apparatus performing gas chromatography on the sample of Table 2;

Figure 2 is the graph obtained by Mass Spectrometry on the first sample after acid hydrolysis and shows presence of cocaine;

Figure 3 is the Mass Spectrum of the same first sample after treatment according to the present invention and shows presence of benzoylecgonine;

Figure 4 is the Mass Spectrum of the same first sample after treatment according to the present invention and shows presence of morphine;

Figure 5 is a chromatogram similar to that of Figure 1 and refers to the first sample treated according to this invention as shown in Table 3;

Figure 6 is similar to the MS of Figure 3 showing presence of benzoylecgonine;

Figure 7 is similar to the MS of Figure 3 showing presence of morphine;

Figure 8 is similar to Figure 1 but relates to a second sample;

Figure 9 is similar to Figure 5 but relates to a second sample;

Figures 10 and 11 are respectively similar to Figures 8 and 9 but relate to a

third sample;

Figures 12 and 13 are respectively similar to Figures 8 and 9 but relate to a fourth sample;

Figure 14 is a diagram showing transformation of cocaine into its metabolite benzoylecgonine;

Figure 15 is a graph showing concentration of cocaine versus concentration of OH^- when the reaction of transformation of cocaine into benzoylecgonine occurs at 100° C for 1 hour; and

Figure 16 is similar to Figure 15 and shows the case in which the reaction occurs at 150°C for 1 hour.

Detailed description of invention embodiments

The process of this invention substantially is a process for the quantitative extraction and transformation of cocaine into benzoylecgonine and for the extraction of similar alkaloids, in particular toxic substances of abuse and/or drugs, which are present in a sample, prepared starting from a solid material.

In the following detailed example, the sample is obtained starting from finely divided hair.

The procedure allows, in particular, the dosage of cocaine by a screening type technique, at the same time allowing the dosage of possible other toxic substances and the like present in the hair, by using kits of reagents available in commerce and intended for the analysis in urine.

The following steps are carried out:

- crush hair in fragments of two-three mm length;
- weigh the fragments to form a sample having a weight of 50 to 300 mg;
- wash the sample with methanol in a closed test tube at room temperature to eliminate possible external substances that might interfere with the results, such as, for example, traces of drugs external to the hair that have deposited on it because of its presence in the air (by so doing, it is possible to distinguish if the hair was that of a handler or that of the consumer of the drug);
-Repeat the step of washing with methanol, if necessary;
- wash the sample with ethanol in a closed test tube to eliminate traces of methanol or water;
- dry in an oven at about 45°C under flow of inert gas, such as, nitrogen;
- add into the test tube 0.5 to 2 ml of the reagent as defined above and shake if necessary;
- heat the test tube to 100°C and maintain at this temperature for 1 hour, e.g. by means of a thermostatic bath or an oven;
- cool the test tube to room temperature, e.g., by immersing the tube into cold water or simply leaving it at room temperature for a suitable time span;
- take the liquid and pour into a test tube of the kind used for urine examination (if necessary, centrifuge the test tube to eliminate turbidity);
- insert the tube in a "first-level screening apparatus" for the quantitative determination of the substances sought for (benzoylecgonine, morphine, methadone, etc);
- adjust settings of the first-level apparatus in a way suitable for small amounts (this may be necessary if the apparatus is alternatively used for determination in hair or urine);
- perform screening –type analyses using the reagents provided with the kit normally employed for the examination of urine;
- read data resulting from the first-level analyses and establish positivity or negativity with reference to the cut-off value.

The above procedure may then be complemented with the following additional steps when confirmation analyses are required:

- arrange the samples in the order of increasing concentration of the sought for substances, determined as above described;
- perform confirmation analyses by analyzing the samples taken in the order of the arrangement (In this way the above mentioned problems of dragging traces of drugs from one sample to the other are overcome).

The invention process provides for the use of a reagent (hereinafter referred to as VMA) which is a buffer solution. The buffer serves for transforming the cocaine present in the hair into its metabolite benzoylecgonine, as shown in the scheme of Figure 14. Cocaine, in the presence of hydroxyl groups and at a suitable temperature first is transformed into an intermediate product and then into benzoylecgonine plus methanol.

Buffer solutions are obtained by reacting a salt with its weak base. These solutions have a stable pH; therefore the VMA reagent is able to produce hydroxyl groups in a steady way. The use of solutions in which the production of hydroxyl groups is not regular creates problems when the cocaine concentration is close to the cut-off limit.

As said above, the composition of the buffer for use in the process is preferably the following:

$$VMA = 0.2\ M\ (NH_4)_2HPO_4 + 5ml/L\ 25\%\ NH_4OH$$

Alternatively, VMA may be replaced by solution in which the component being the source of hydroxyl groups is selected among the following non-limitative list of substances: aluminum hydroxide, barium hydroxide octahydrate, benzyltriethylammonium hydroxide, benzyltrimethylammonium

hydroxide, calcium hydroxide, phenylhydrargirium hydroxide, lithium hydroxide, lithium hydroxide monohydrate, magnesium hydroxide, potassium hydroxide, potassium hydroxyantimoniate, sodium hydroxide, sodium hydroxide monohydrate, strontium hydroxyde octahydrate, tetramethylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide.

As solvent, any of the following can be used in alternative:
ethanol, methanol, water, monobasic ammonium phosphate, ammonium acetate, ammonium benzoate, ammonium bicarbonate, ammonium bichromate, ammonium bisulphate, ammonium bromide, ammonium carbamate, ammonium carbonate, ammonium citrate bibasic, ammonium chromate, ammonium iodide, molibdate, ammonium monovanadate, ammonium nitrate, ammonium oxalate monohydrate, ammonium persulphate, ammonium sulphate, ammonium sulphamate, ammonium sulphite, ammonium sulphide, ammonium tartrate, ammonium thiocyanate, ammonium thioglycolate, ammonium thiosulphate, ammonium chloride, sodium phosphate monobasic, sodium phosphate bibasic, potassium phosphate monobasic, potassium phosphate bibasic.

Figure 15 shows a graph in which the percentage of cocaine transformed into benzoylecgonine is reported versus the concentration of OH^- when the reaction is carried out at the temperature of 100°C for 1 hour. From the graph it appears evident that the transformation is maintained at high levels (at least 70%) for a range of OH^- concentrations of from 0.03 to 0.5 M.

From the graph of Figure 16, which shows hydroxyl concentrations after, respectively, 0, 15, 30 and 60 minutes in a reaction carried out at 150°C; it appears evident that the percentage of transformed cocaine is high only around the abscissa point of 15 minutes.

From all of the above and numerous other experiments the optimal values for the reaction temperature and time result to be, respectively, 100°C and 1 hour.

The examples reported below show the quantitative determination of cocaine in hair performed both with known techniques and with the process of the invention. Analyses have been carried out using the following instruments: for the screening analyses, the ROCHE instrumentation named "COBAS MIRA PLUS" which uses reagents provided by the same company and named "ABUSCREEN ON LINE"; for GC/MS the instrument provided by the company VARIAN which is named "SATURN GC/MS", model 4D.

EXAMPLES

Before proceeding to the various screening analyses the apparatus has been controlled with a sample positive to both cocaine and morphine in order to verify feasibility of the methodology.

As can be seen from Table 1 the sample resulted positive to both cocaine and morphine:

TABLE 1

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.16 ng/ml	POSITIVE
COCAINE	0.15 ng/ml	POSITIVE

The concentration values detected by the apparatus resulted to be 0.16 for morphine and 0.15 for cocaine, in line with the expected values for both substances present in the control sample.

EXAMPLE 1

In this example a sample has been analyzed which resulted, in the end, to be positive to both cocaine and morphine.

The sample was subjected to conventional analysis with acid hydrolysis and then subjected to screening analysis which gave the following results:

TABLE 2

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.13 ng/ml	POSITIVE
COCAINE	0.01 ng/ml	NEGATIVE

As can be seen, acid hydrolysis was able to extract an amount of morphine higher than the limit, but the amount of cocaine resulted to be too little to allow detection by the screening apparatus. The reason is that cocaine was extracted as such and not transformed into its metabolite benzoylecgonine, which latter is just what present screening methods detect.

The same sample was then subjected to confirmatory analysis by GC/MS with the results reported in Figures 1 to 4. Specifically, from Figure 1 it can be seen that the chromatogram of the first sample shows a high peak at the position of cocaine and a very small peak at the position of benzoylecgonine. The same Figure 1 also shows confirmation of the presence of morphine.

Additional confirmations of the presence of both cocaine and morphine result from the graphs of Figures 2, 3 and 4.

The sample of Example 1 was then subjected to the procedure of this invention to give the results shown in Table 3.

TABLE 3

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.23 ng/ml	POSITIVE
COCAINE	0.37 ng/ml	POSITIVE

It is immediately clear that the values detected for both morphine and cocaine are higher than the respective limits, therefore the sample is positive for both.

The high value detected for cocaine is to be ascribed to the benzoylecgonine that has been produced during the transformation reaction.

The same sample of Example 1 treated with the process of this invention has been subjected to GC/MS confirmation analyses with the results reported in Figures 5 to 7. As can be seen from Figure 5, the cocaine peak practically cannot be seen anymore because cocaine has been completely transformed in benzoylecgonine, the peak of which is well apparent in the same Figure 5.

EXAMPLE 2

Again in this example a sample which, in the end, resulted to be positive to both cocaine and morphine has been analyzed first by the conventional analytical method of acid hydrolysis and screening with the results reported in Table 4.

TABLE 4

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.32 ng/ml	POSITIVE
COCAINE	0.03 ng/ml	NEGATIVE

Like in the preceding Example, acid hydrolysis was able to extract an amount of morphine higher than the limit, but the quantity of cocaine extracted was too little to allow detection by the apparatus.

The sample was then subjected to GC/MS confirmatory analyses to give the results reported in Figure 8 where it can be seen that the chromatogram shows a high peak at the position of cocaine and a negligible peak at the position of benzoylecgonine.

The sample of Example 2 was then subjected to the invention process and to screening analyses with the results reported in Table 5.

TABLE 5

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.28 ng/ml	POSITIVE
COCAINE	0.99 ng/ml	POSITIVE

Both the cocaine and the morphine values are higher than the respective limits, therefore the sample is positive to both.

The sample of this Example, treated according to the invention process, was also subjected to GC/MS confirmation analyses with the results reported in Figure 9 where it can be seen that the cocaine peak is not practically present anymore whereas the benzoylecgonine peak is high.

EXAMPLE 3

In this Example a sample has been analyzed which, at the end, resulted to be positive to cocaine only and not to morphine.

This sample was first subjected to acid hydrolysis and screening by the conventional methods to give the results reported in Table 6.

TABLE 6

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.02 ng/ml	NEGATIVE
COCAINE	0.01 ng/ml	NEGATIVE

By acid hydrolysis a quantity of morphine lower than the limit was extracted. The quantity of cocaine was also too little to allow the equipment to detect it. By using the methods known in the art, therefore, nothing can be said as to the positivity or negativity of the sample and it was necessary to make recourse to the GC/MS confirmation analyses that gave the results reported in Figure 10. In such Figure, the chromatogram of the sample of this Example shows a high peak at the position of cocaine and a negligible peak at the position of benzoylecgonine. This confirms once more that acid hydrolysis extracted cocaine as such without any transformation.

The same sample of this Example has also been treated according to the invention process and subjected to screening analysis. Results are reported in Table 7.

TABLE 7

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.03 ng/ml	NEGATIVE
COCAINE	0.13 ng/ml	POSITIVE

It is immediately evident that while the value of morphine is lower than the limit, that of cocaine is slightly higher than the cut-off and therefore the sample is only positive to cocaine.

This same sample, after treatment with the invention process, has been subjected to GC/MS confirmation analyses with the results shown in Figure 11. In this Figure, the peak of cocaine has practically disappeared because the same was completely transformed into benzoylecgonine, the peak of which is, instead, very high and well apparent.

EXAMPLE 4

This fourth Example also analyzes a sample which, at the end, resulted positive to cocaine and not to morphine.

Analyses were carried out by applying the same scheme as in the preceding Examples.

Table 8 shows that upon analysis with acid hydrolysis the sample seems to be completely negative to both morphine and cocaine, whereas Figure 12 indicates the presence of cocaine in an amount higher than the limit.

TABLE 8

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.02 ng/ml	NEGATIVE
COCAINE	0.05 ng/ml	NEGATIVE

The same sample, treated with the invention process (VMA reagent) already on the first screening analysis shows a presence of cocaine higher than the limit, see Table 9.

TABLE 9

SUBSTANCE	CONCENTRATION	POSITIVITY/NEGATIVITY
MORPHINE	0.02 ng/ml	NEGATIVE
COCAINE	0.64 ng/ml	POSITIVE

As usual, this result was confirmed by GC/MS analysis as shown in Figure 13. The big peak of benzoylecgonine indicates that, before the treatment, the sample contained cocaine in a percentage higher than the cut-off.

The instant application is based upon Italian patent application VR99A000059, filed on 20 July 1999, the disclosure of which is hereby expressly incorporated by reference thereto, and the priority of which is hereby claimed.

CLAIMS

1. A screening-type process for the quantitative determination of cocaine and other alkaloids which are present in a solid sample which includes the steps of:
   - providing a solid sample in a finely divided or powdered form;
   - completely immersing the sample into a liquid reagent providing a constant concentration of hydroxyl groups;
   - maintaining the sample immersed in the liquid at a temperature in a range from 10 to 250°C for a period of time in a range from a few seconds to 48 hours; and
   - analyzing the liquid separated from the solid with a conventional kit for the determination of the said substances in urine.

2. Process according to claim 1, wherein the solid sample is a sample of hair.

3. Process according to claims 1 and 2, wherein said range of temperature is from 100 to 150°C.

4. Process according to claims 1 and 2, wherein said range of period of time is from 15 minutes to 24 hours.

5. Process according to any preceding claims, wherein said temperature is maintained at 100°C for 1 hour.

6. Process according to any of the preceding claims, wherein the concentration of hydroxyl groups is in the range of from 0.0001 to 5 M;

7. Process according to any of the preceding claims, wherein the concentration of hydroxyl groups is in the range of 0.03 to 0.5 M.

8. Process according to any preceding claims, wherein the concentration of hydroxyl groups is in the range of 0.04 to 0.33 M.

9. Process according to any of the preceding claims, wherein the liquid reagent is ammonia buffer.

10. Process according to claim 7, wherein the buffer is 0.2 M (NH4)2HPO4 with the addition of 5 ml of 25% NH4OH to each liter thereof.

11. Process according to any preceding claims, which further comprises the steps of:

- arranging the analyzed samples in the increasing order of concentration of drugs; and
- performing confirmation analyses with standard techniques of the samples taken in the said order.

12. Screening-type process for the quantitative determination of cocaine and other alkaloids which are present in a solid sample, comprising the following steps:

- providing a sample made of about 50 to 300 mg of finely divided and/or powdered material;
- adding in the test tube containing the said sample a suitable liquid reagent until the sample is completely immersed, said reagent being capable of performing extraction and transformation of cocaine into benzoylecgonine and at the same time of extracting other similar substances which are present in the sample
- if necessary, agitating the test tube to facilitate immersion of the sample;
- heating the contents of the test tube to a temperature T1 for a time interval t1 by keeping the test tube immersed in a thermostated bath or by

placing it in an oven;

- cooling the test tube to room temperature;
- taking the liquid and transferring it into a test tube suitable for a screening type instrument;
- performing the screening by using a kit of reagents for the determination of the said substances in urine;
- reading the data resulting from the first level instrumentation to verify the concentration values with respect to the cut-off limit; and
- contemporaneously determining the amount(s) of substance(s) present.

13. Reagent for use in the process of claims 1, 9 or 10, which is a liquid that provides a constant concentration of hydroxyl groups in the range of from 0.04 to 0.33 M.

14. Reagent according to claim 12, which is 0.2 M $(NH_4)_2HPO_4$ with the addition of 5 ml of 25% NH_4OH for each liter thereof.

15. Reagent according to claim 11 or 12, wherein said solution comprises a solute selected among aluminum hydroxide, barium hydroxide octahydrate, benzyltriethylammonium hydroxide, benzyltrimethylammonium hydroxide, calcium hydroxide, phenylhydrargirium hydroxide, lithium hydroxide, lithium hydroxide monohydrate, magnesium hydroxide, potassium hydroxide,potassium hydroxyantimoniate, sodium hydroxide, sodium hydroxide monohydrate, strontium hydroxyde octahydrate, tetramethylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide, tetrapropylammonium hydroxide, trimethylvinylammonium hydroxide, dissolved in a solvent selected among ethanol, methanol, water, monobasic ammonium phosphate, ammonium acetate, ammonium benzoate, ammonium bicarbonate, ammonium bichromate, ammonium bisulphate, ammonium bromide, ammonium

carbamate, ammonium carbonate, ammonium citrate bibasic, ammonium chromate, ammonium iodide, molibdate, ammonium monovanadate, ammonium nitrate, ammonium oxalate monohydrate, ammonium persulphate, ammonium sulphate, ammonium sulphamate, ammonium sulphite, ammonium sulphide, ammonium tartrate, ammonium thiocyanate, ammonium thioglycolate, ammonium thiosulphate, ammonium chloride, sodium phosphate monobasic, sodium phosphate bibasic, potassium phosphate monobasic, potassium phosphate bibasic.

16. Use of the reagent according to claims 12 to 15 for the carrying out of the process of any of claims 1 to 10 and 11.

17. Diagnostic kit including the reagent of claim 12 as one of its components.

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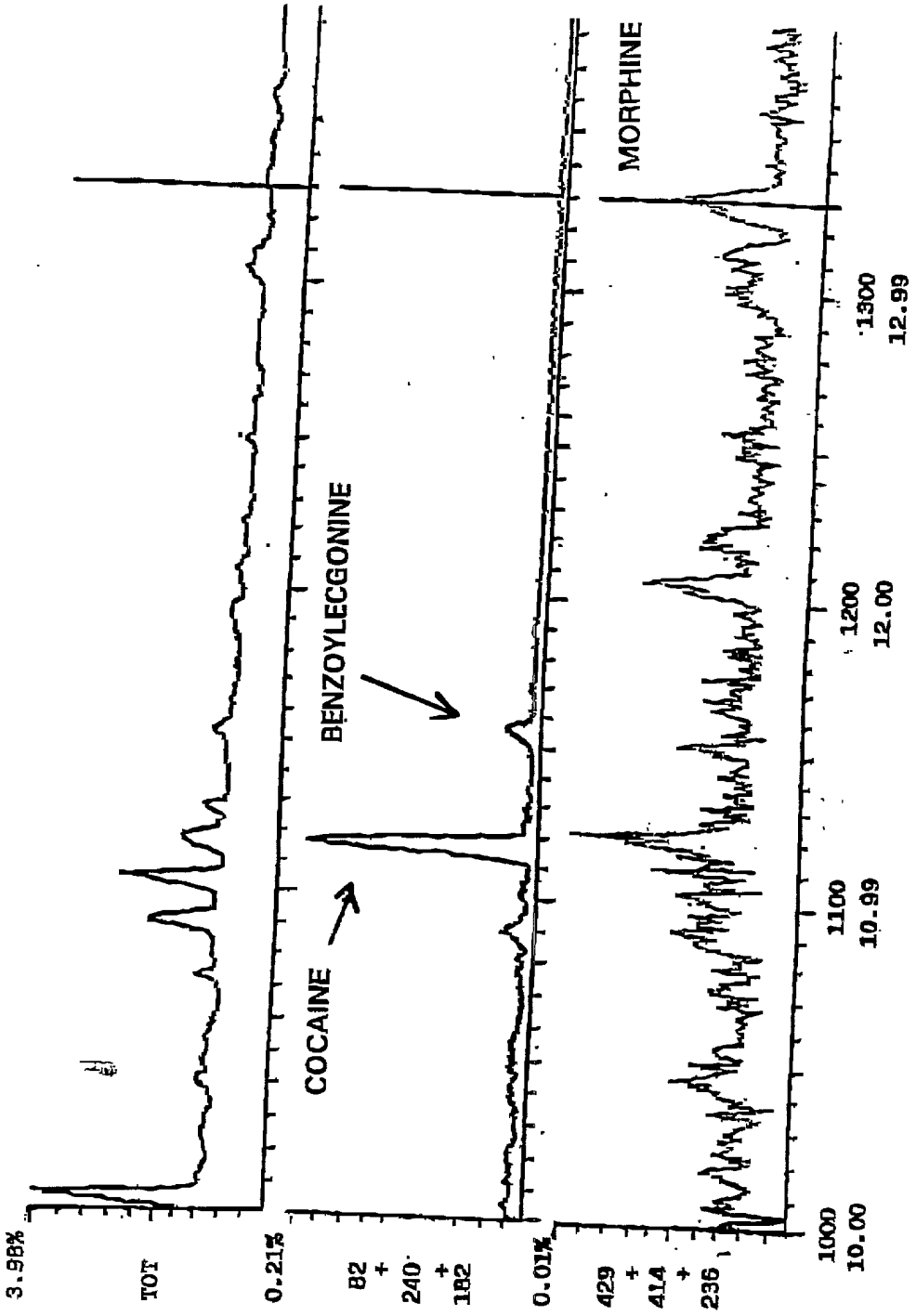

Fig. 1

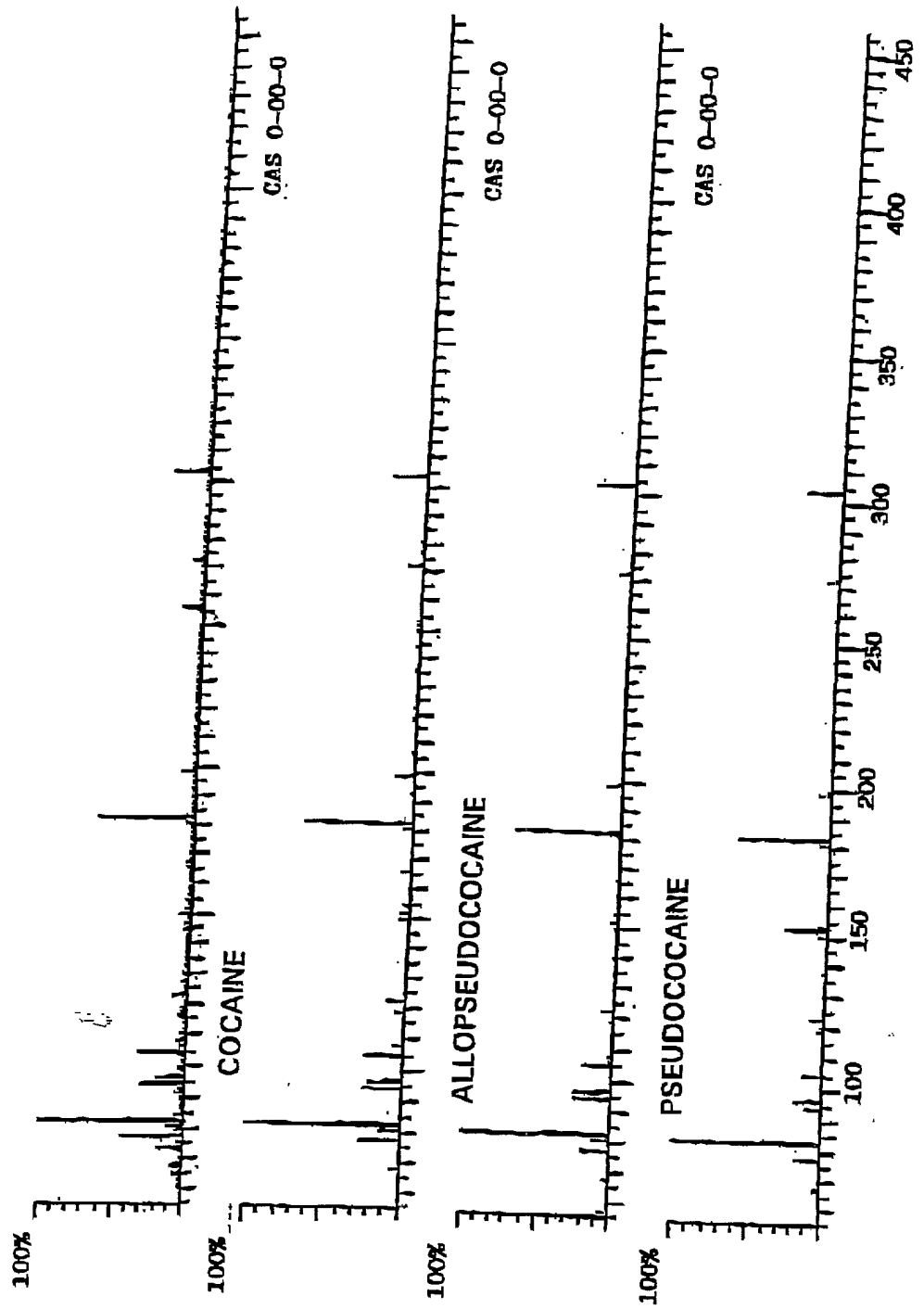
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Fig. 2

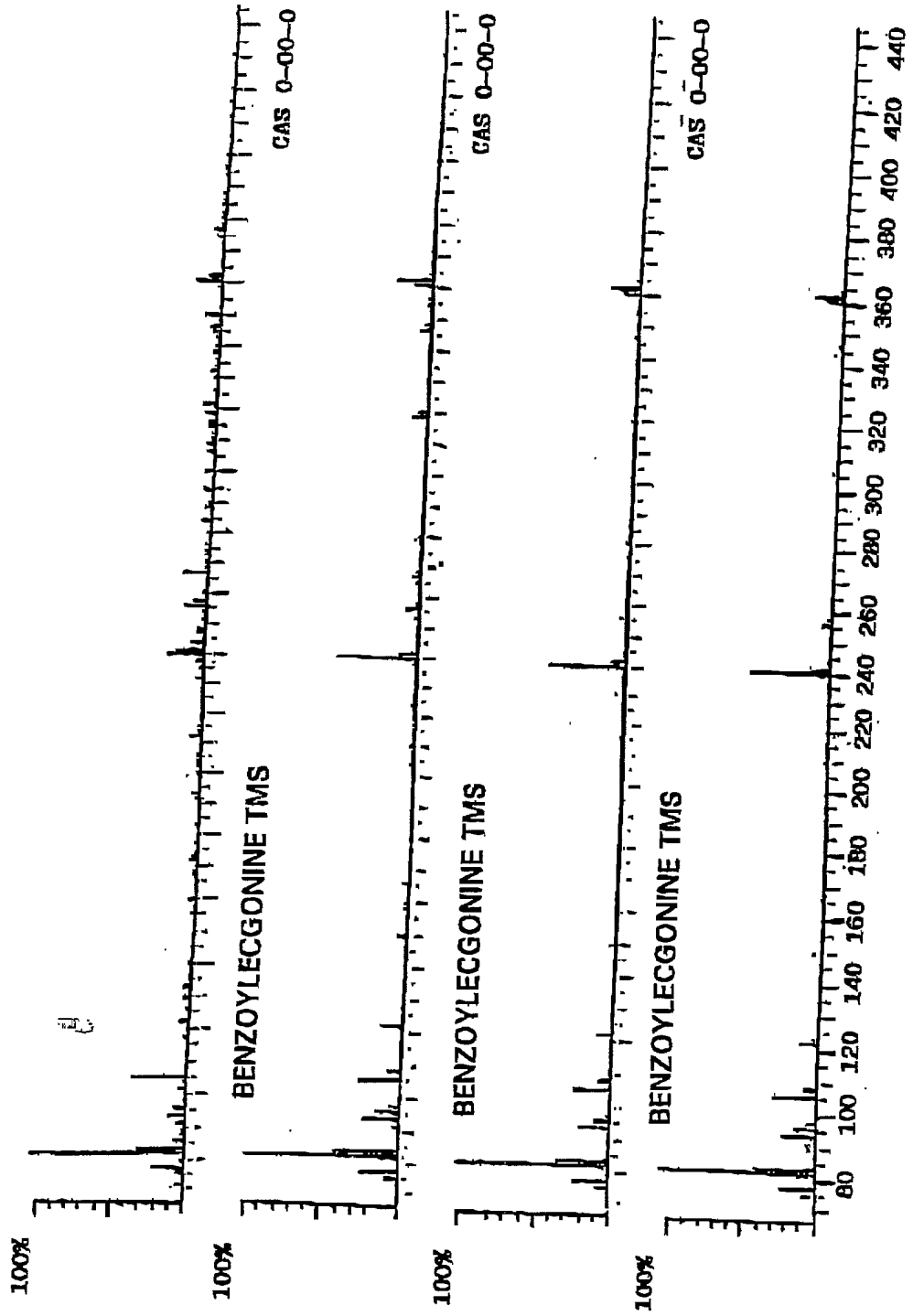

Fig. 3

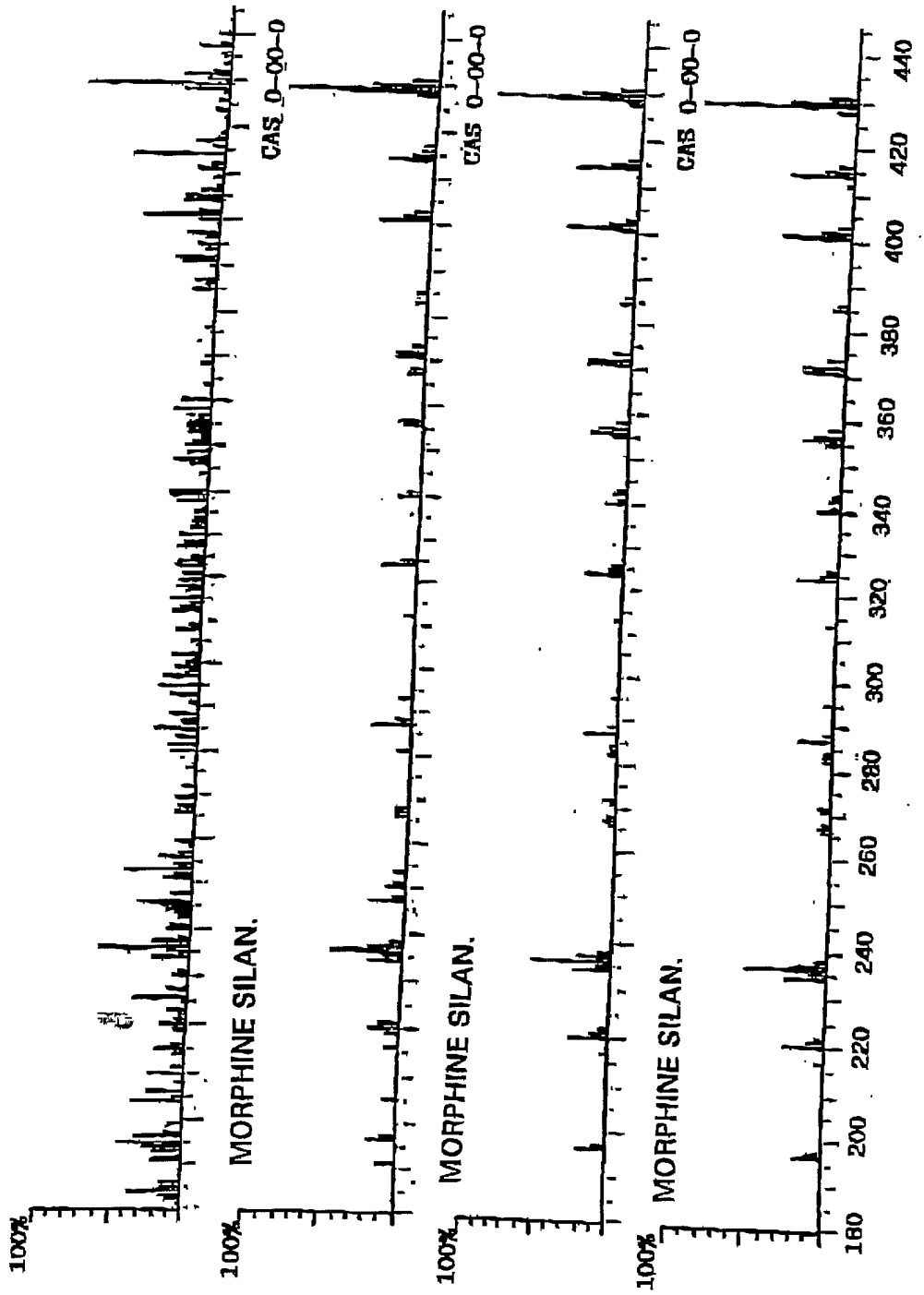
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Fig. 4

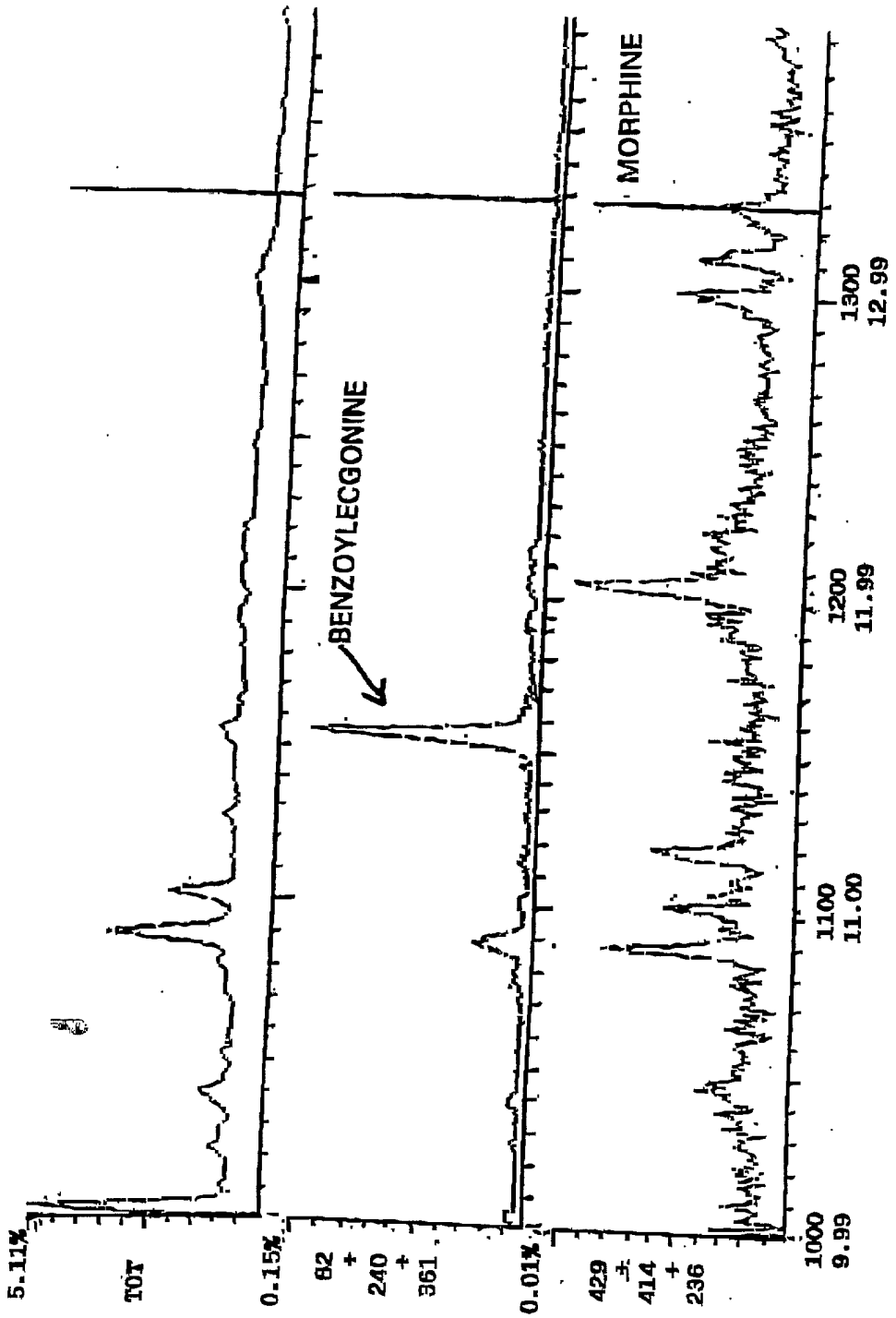
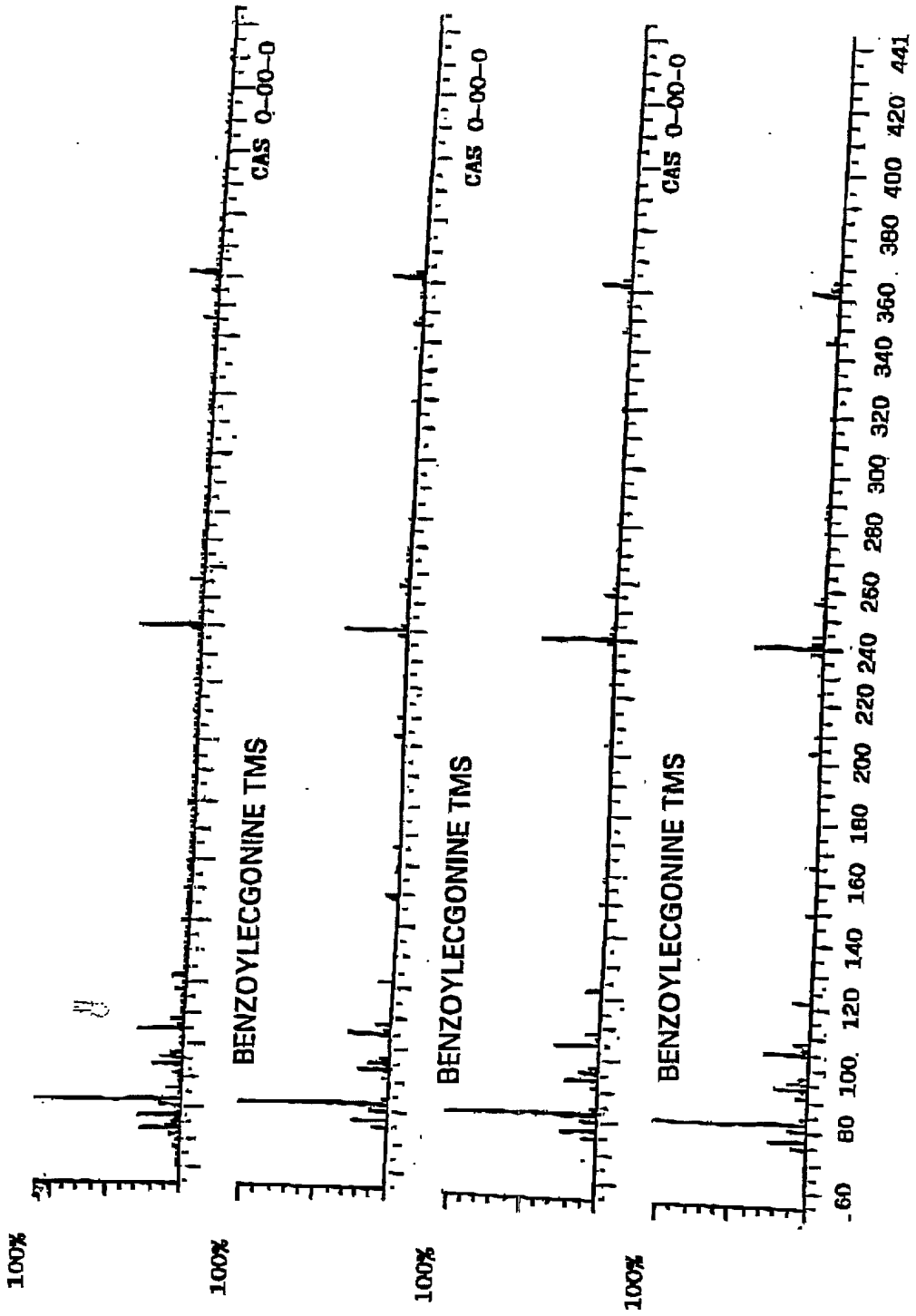


Fig. 5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CAS 0-00-0
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BENZOYLECGONINE TMS
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Fig. 6

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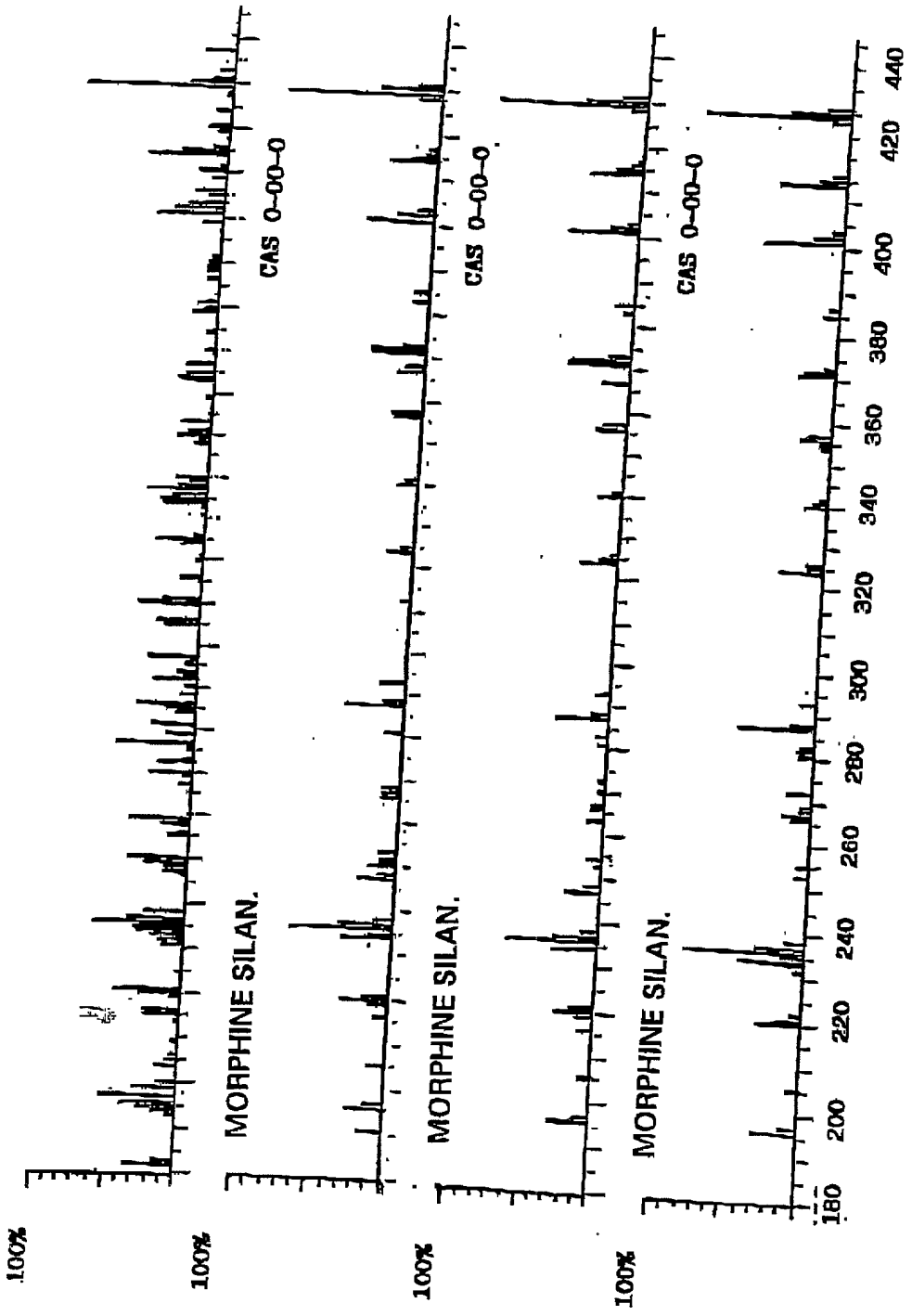


Fig. 7

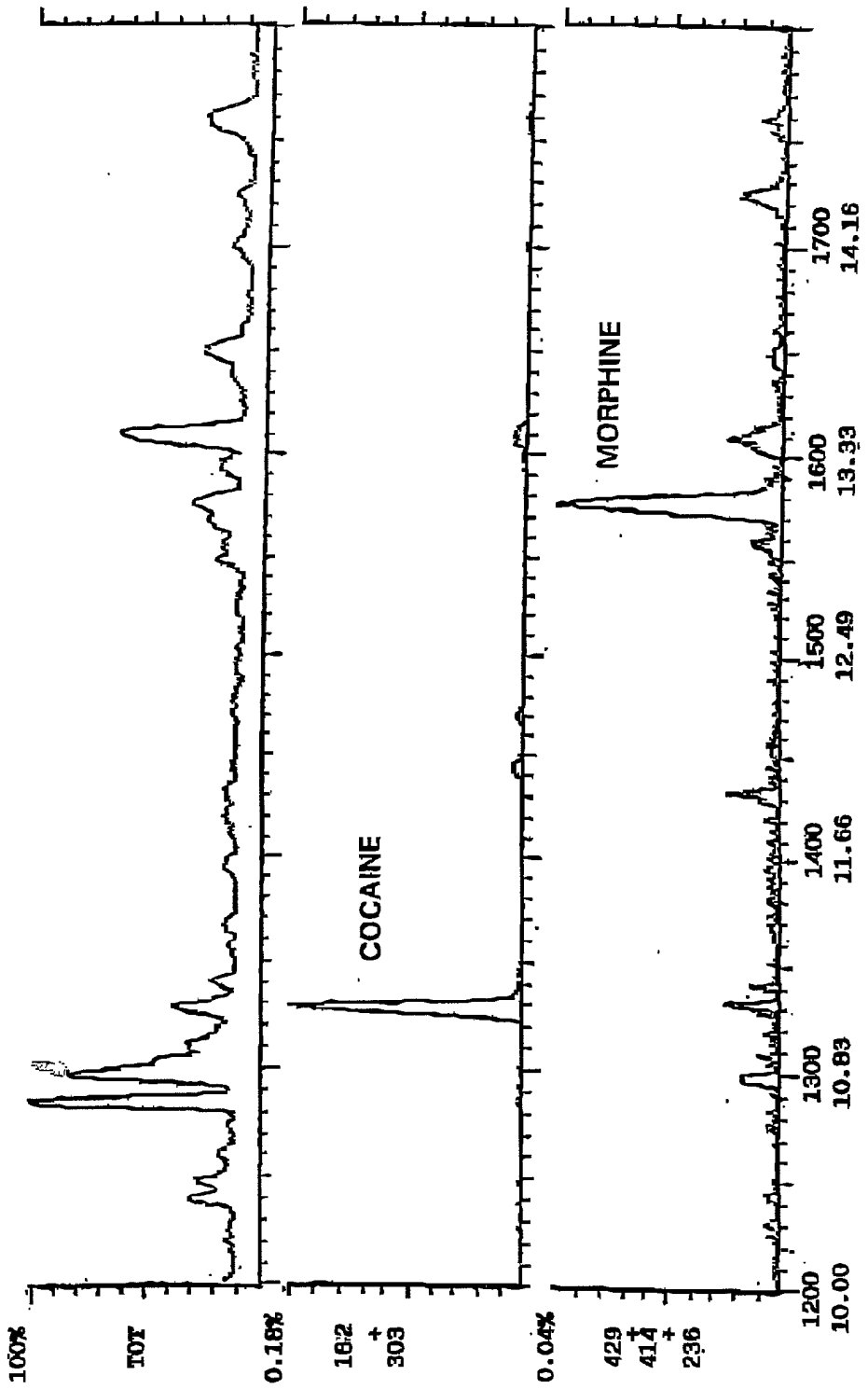
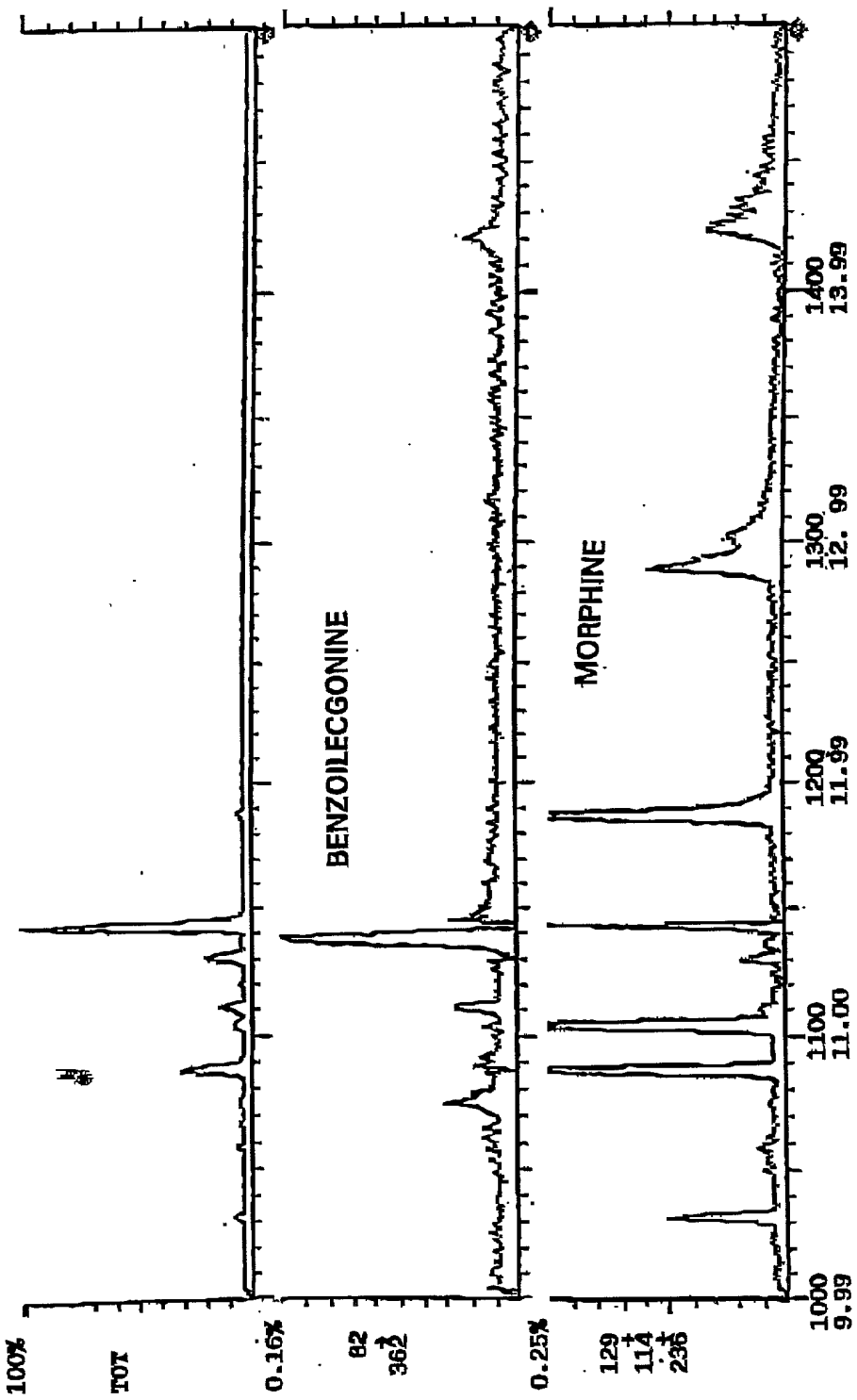


Fig. 8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0.25%
129
114
236
1000
9.99
1100
11.00
1200
11.99
1300
12. 99
1400
13.99

Fig. 9

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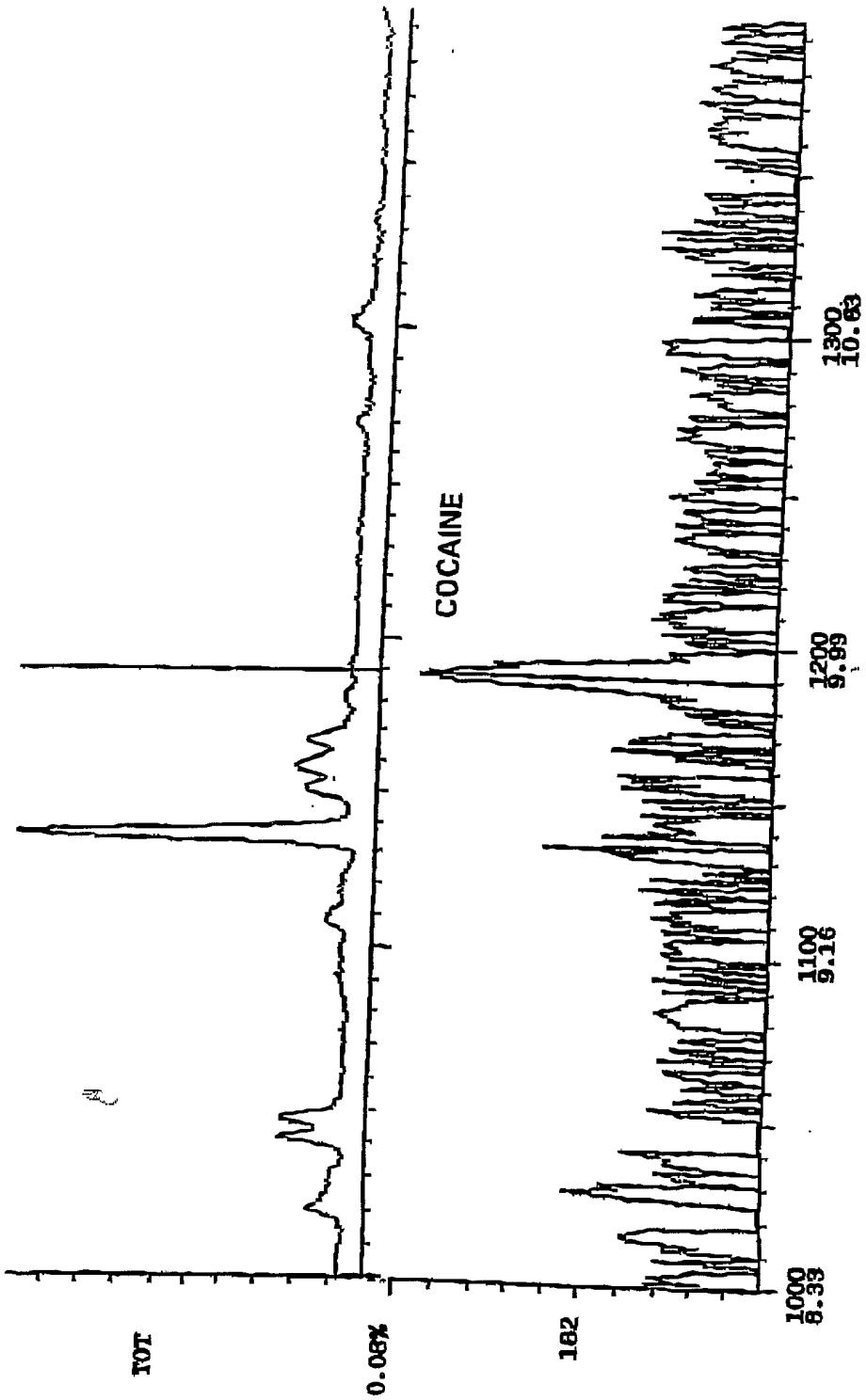

Fig. 10

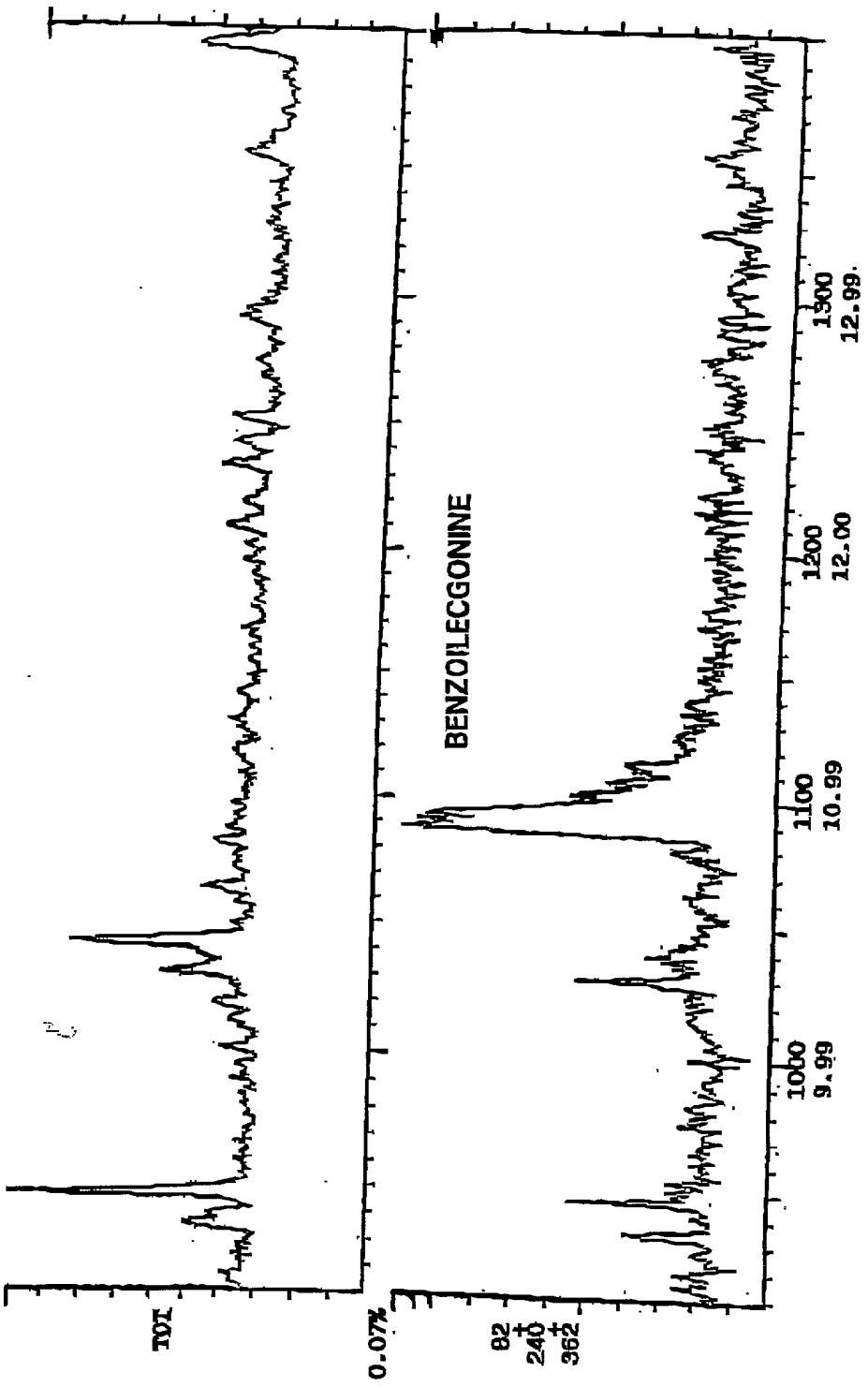

Fig. 11

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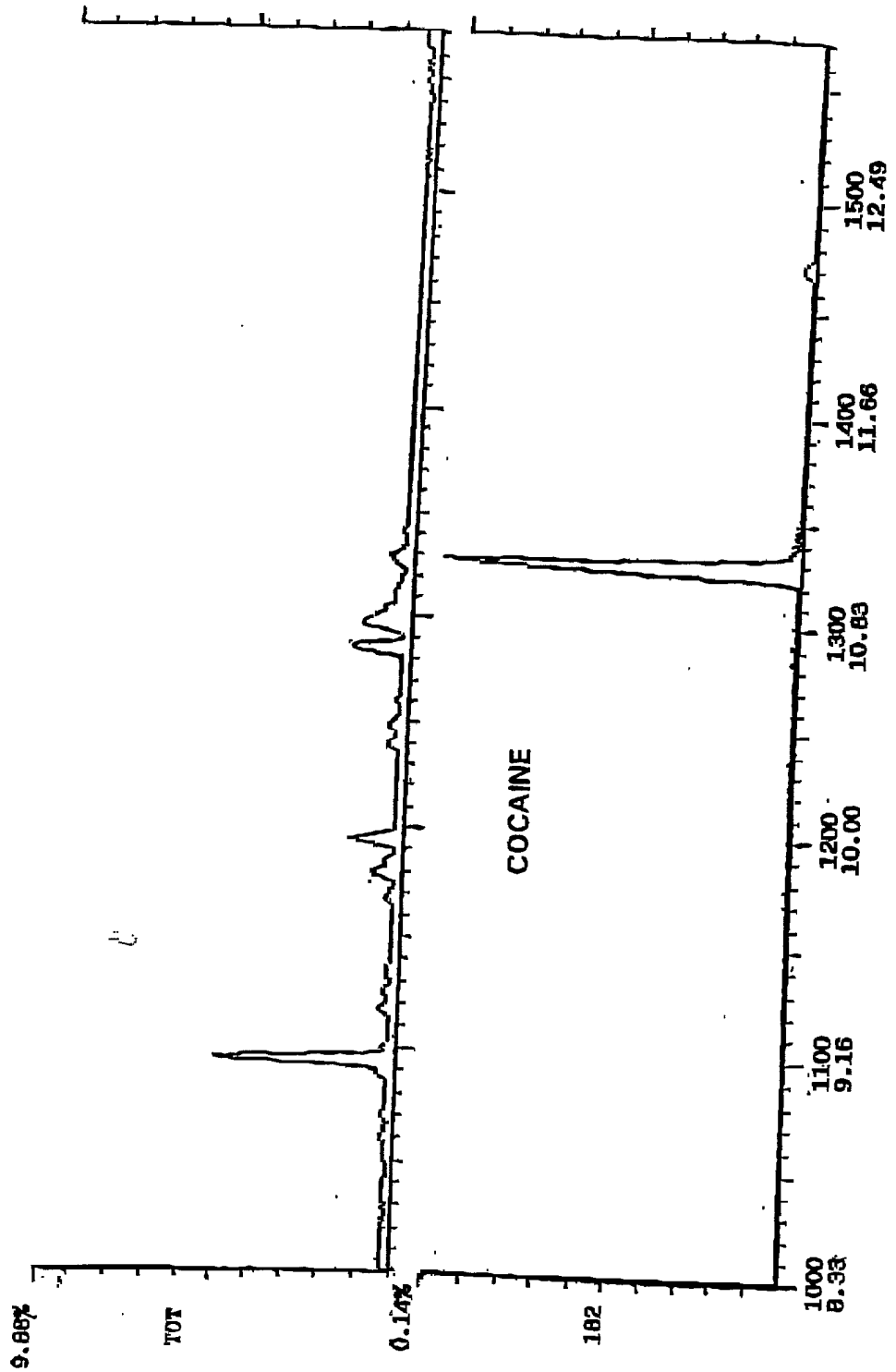

Fig. 12

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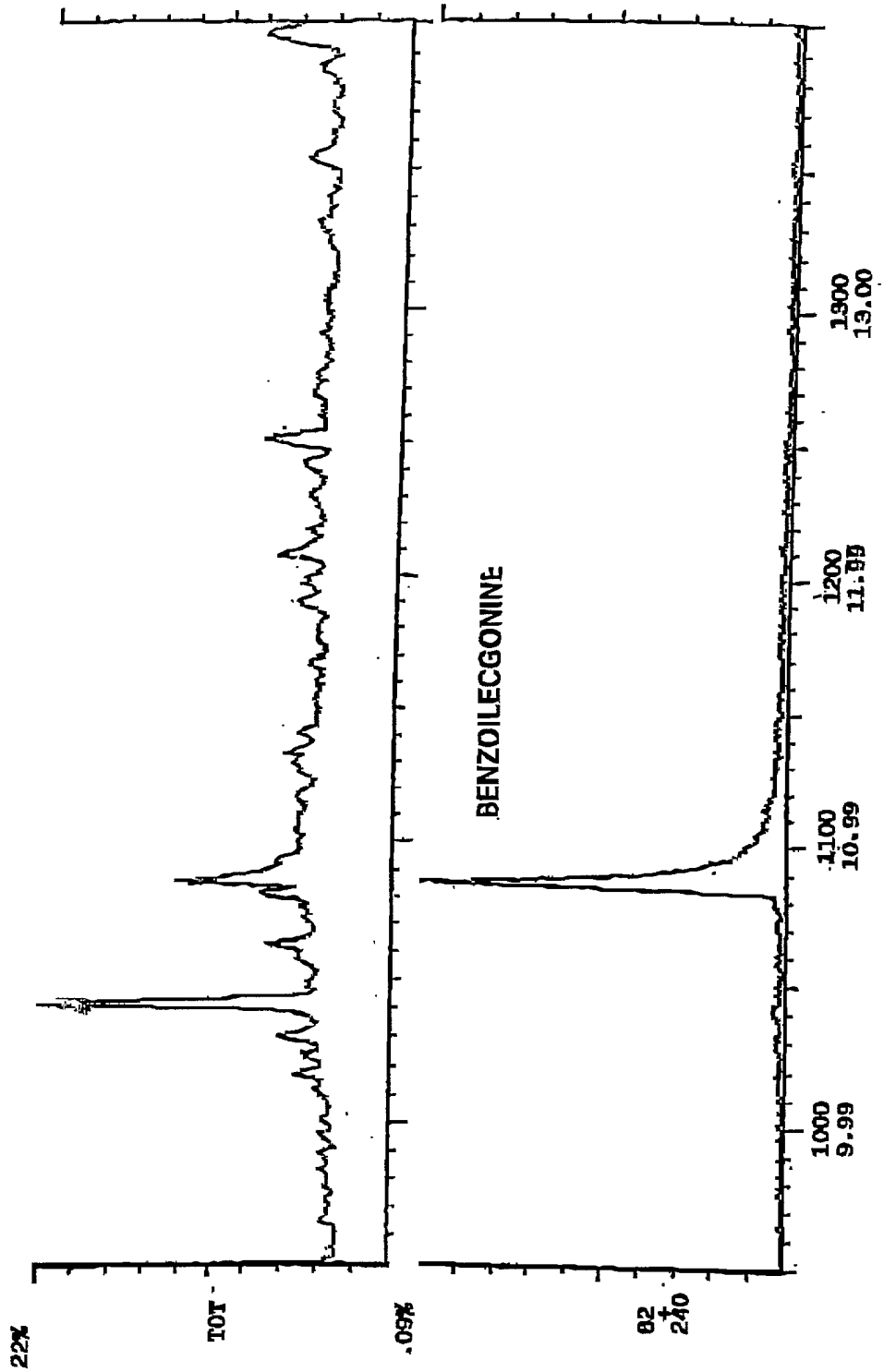

Fig. 13

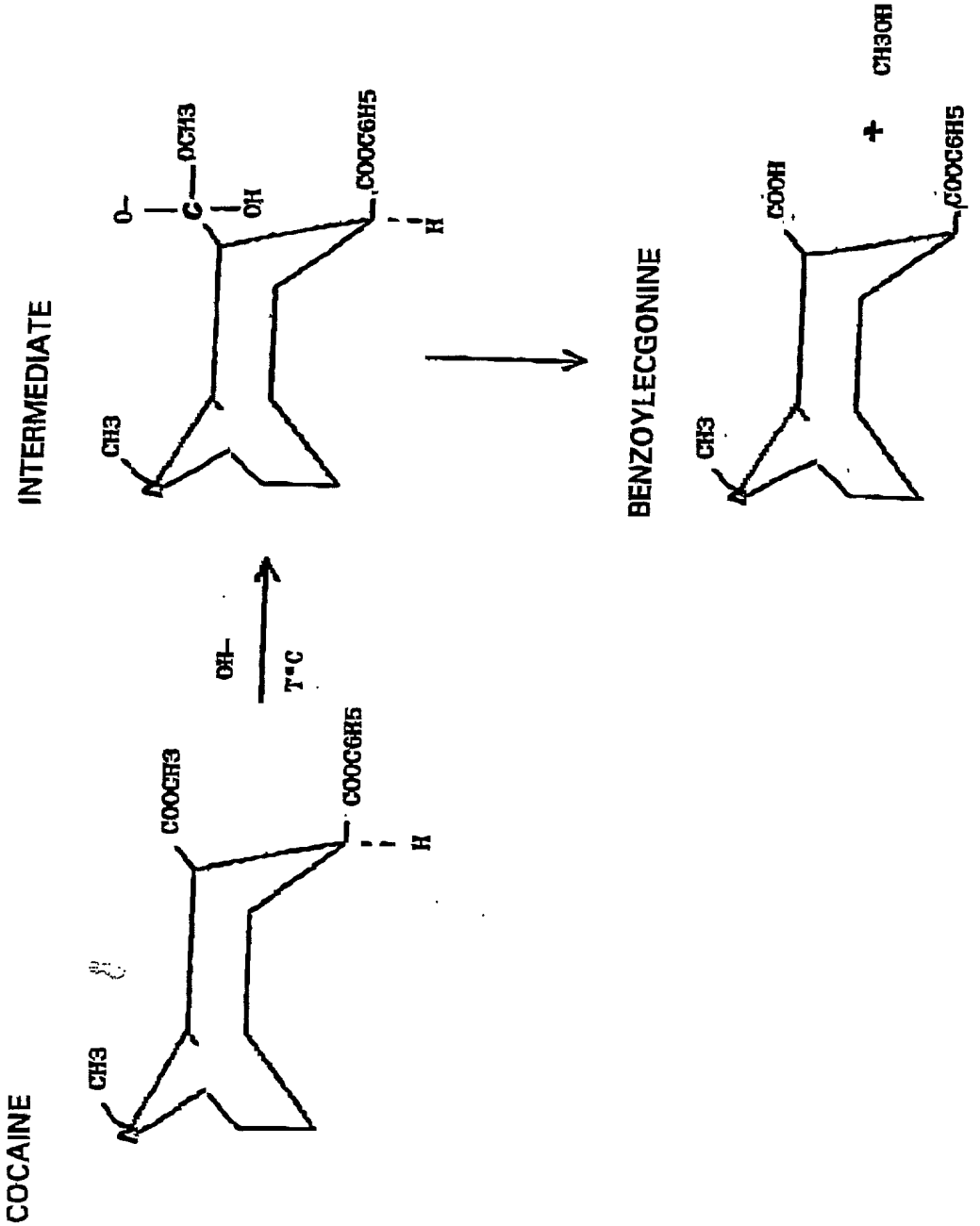
INTERMEDIATE
BENZOYLECGONINE
COCAINE
OCH3
OH
COOC6H5
H
CH3
COOH
CH3OH
+
OH-
T°C

Fig. 14

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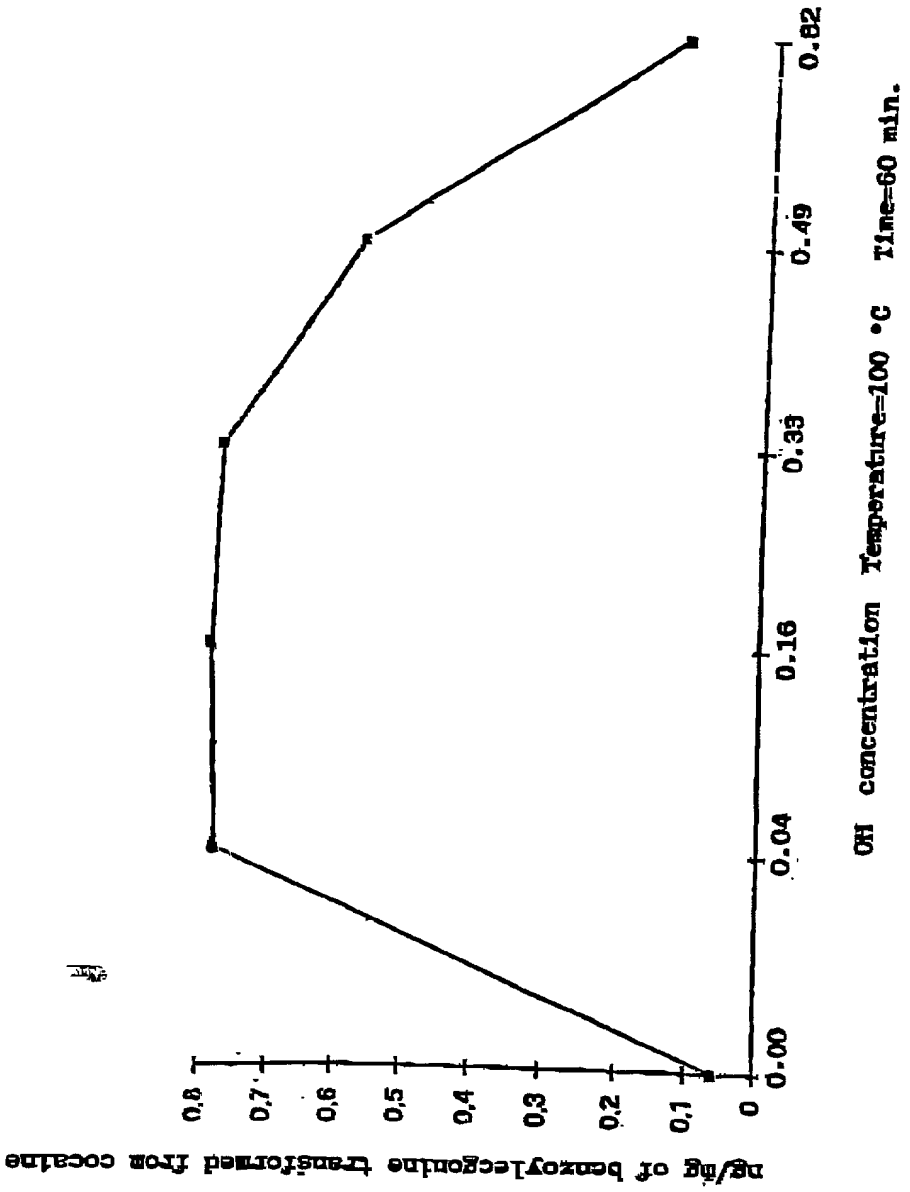

Fig. 15

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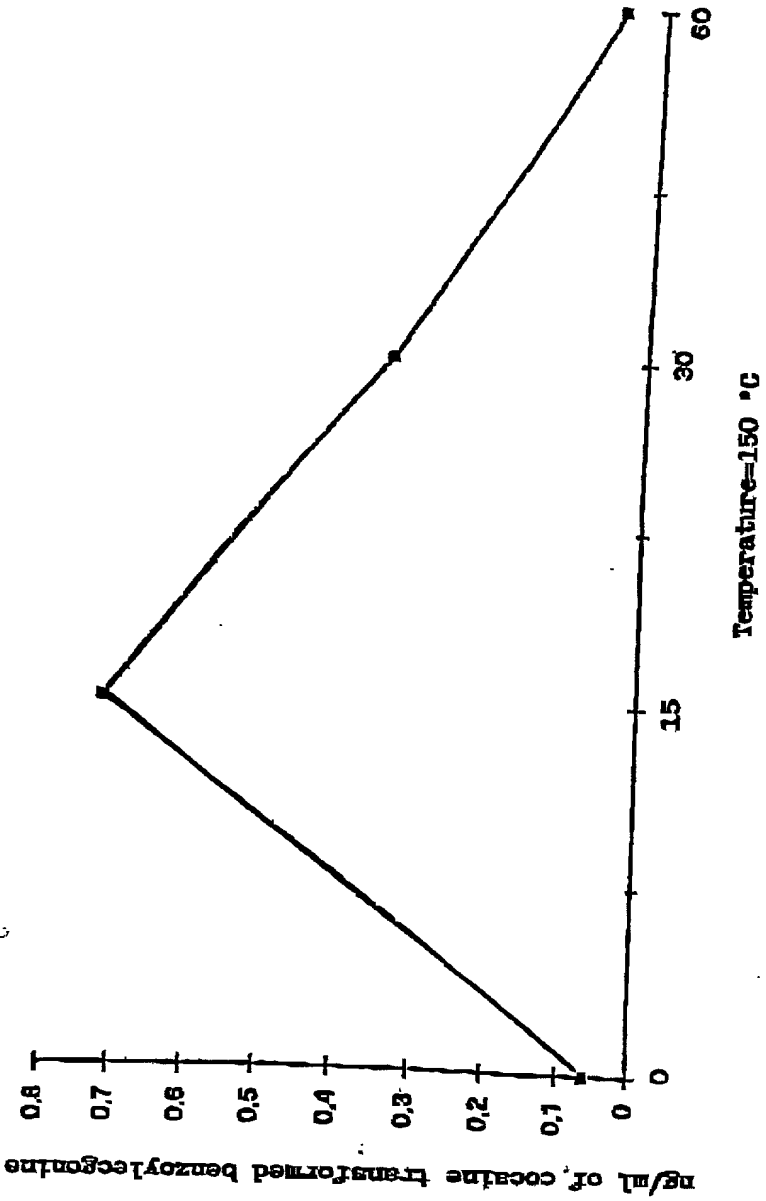

Fig. 16

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I (we) hereby declare that:

My residence, post office address and citizenship are the same as stated below next to my name.

I (we) believe I am (we are) an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROCESS FOR THE QUANTITATIVE DETERMINATION OF ALKALOIDS SUCH AS COCAINE IN A SOLID SAMPLE AND REAGENT FOR USE IN SUCH PROCESS

the specification of which (check one)

[x] Is attached hereto.

[ ] Was filed on _____ as Application Serial No. _____

and was amended on _____ (if applicable).

[ ] was filed as PCT International application No. _____ on _____

and was amended on _____ (if applicable).

I (we) hereby state that I (we) have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I (we) acknowledge the duty to disclose information known to me to be material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I (we) hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed

Number	Country	Filing Date (mm/dd/yyyy)	Priority Claimed
VR99A000059	ITALY	20/07/1999	[X] Yes [ ] No
PCT/IB00/00966	PCT	14/07/2000	[X] Yes [ ] No

I (we) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Number	Filing Date (mm/dd/yyyy)

I (we) hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulation, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

Application Serial No.	Filing Date (d/m/y)	Status (Patented, Pending, Abandoned)

I (we) hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I (we) hereby declare that all statements made herein of my (our) own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Date Inventor's Signature

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15 GEN. 2002 _____
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15 GEN. 2002 _____
Date Inventor's Signature